Chemoenzymatic Synthesis of Chiral Organosulfur Compounds

by

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DECLARATION

I declare that this thesis, except where otherwise stated, is based on my own research carried out in the School of Chemistry, The Queen's University of Belfast, between October 1997 and Ocober 2000.

Signed: ……..

To my family and to the memory of James David Ballantine.

Abstract

This thesis is primarily concerned with the enzyme- catalysed synthesis of sulfoxides using reductase and dioxygenase enzymes.

Chapter 1 provides an introduction to the topic of redox chemistry with particular emphasis on the application of reductase and dioxygenase enzymes in organosulfur chemistry. Earlier literature methods for the production of enantiopure sulfoxides are reviewed. A brief discussion of the methods used for the determination of enantiomeric excess and absolute configuration is provided.

Chapter 2 contains results obtained using a range of whole-cell bacteria each using a dimethyl sulfoxide reductase enzyme. The synthesis of a series of racemic sulfoxides and the development of appropriate CSPHPLC analytical methods is discussed. Kinetic resolutions of a series of sulfoxides have been achieved.

Chapter 3 contains a presentation of results using dioxygenase enzymes as biocatalysts for the asymmetric sulfoxidation of dialkyl sulfoxides including thioacetal sulfoxides. A new range of monosulfoxides, *cis*-dihydrodiols and *cis*-dihydrodiol sulfoxides have been isolated in enantiopure form.

Chapter 4 is focussed on the application of chiral sulfoxides in synthesis. A new chemoenzymatic route to diol sulfoxide enantiomers and the derived enantiopure phenols and catechols is discussed. The application of chemically synthesised sulfoxide enantiomers in the production of hydroxy sulfoxides is reported.

Chapter 5 provides a full experimental section where the synthesis of sulfides and racemic sulfoxides is included. The methods used in the isolation and characterisation of bioproducts from the biotransformation are discussed and full experimental details given.

Chapter 1. Introduction. Page no.

Abbreviations**.**

Ar	aryl
bp	boiling point
BPDO	biphenyl dioxygenase
br	broad
BZDO	benzene dioxygenase
CD	circular dichroism
$CHCl_3$	chloroform
CSP	chiral stationary phase
d	doublet
dd	doublet of doublets
DMD	dimethyl dioxirane
DMF	dimethylformamide
dt	doublet of triplets
DMSO	dimethyl sulfoxide
ee	enantiomeric excess
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
GC	gas chromatography
^{1}H-NMR	proton magnetic resonance spectroscopy
hr	hours
HPLC	high performance liquid chromatography
IPA	*iso*-propylalcohol
IR	infrared spectroscopy
m	multiplet
*M*CPBA	*meta*-chloroperoxybenzoic acid
MDBBA	2-(1-methoxy-2,2-dimethylpropyl)benzene boronic acid
Me	methyl
MEBBA	2-(1-methoxyethyl)benzene boronic acid
MeOH	methanol
min	minutes
MMBBA	2-(1-methoxy-2-methypropyl)benzene boronic Acid
mp	melting point
MPBBA	2-(1-methoxy-1-phenylmethyl)benzene boronic acid
MS	mass spectrometry
MTBBA	2-(1-methoxy-2,2,2-trifluoroethyl)benzene boronic acid
MTPA	α-methoxy-α-(trifluromethyl)phenylacetic acid
NDD	naphthalene *cis*-dihydrodiol dehydrogenase
NDO	naphthalene dioxygenase
PAH	polycyclic aromatic hydrocarbon
Ph	phenyl
PLC	preparative thin layer chromatography
r.t.	room temperature

s	singlet
t	triplet
TDO	toluene dioxygenase
TFAA	trifluoroacetic ahydride
THF	tetrahydrofuran
TLC	thin layer chromatography

Chapter 1. Introduction.

Enzymes have been utilised by man in the production of food and drink for thousands of years. Even before the industrial revolution, the use of enzymes, found in micro-organisms, had been refined and perfected in techniques such as brewing, baking and cheese manufacture to name but a few applications. Nowadays it is possible to manipulate a whole series of micro-organisms into metabolising a range of organic chemicals. What information and benefits will the study of enzyme metabolism of organic compounds provide?

- it explains how nature can dispose of xenobiotic compounds,
- it shows the potential of enzymes in synthesis,
- the chiral bioproducts provide a valuable addition to the 'chiral pool'.

This introductory chapter will discuss certain aspects of metabolism of xenobiotics and compare the synthetic potential of some metabolic enzymes with their chemical equivalents, in relation to the metabolism of mainly organosulfur and aromatic compounds. It will also highlight possible uses for the metabolites.

1.1. Chemoenzymatic synthesis.

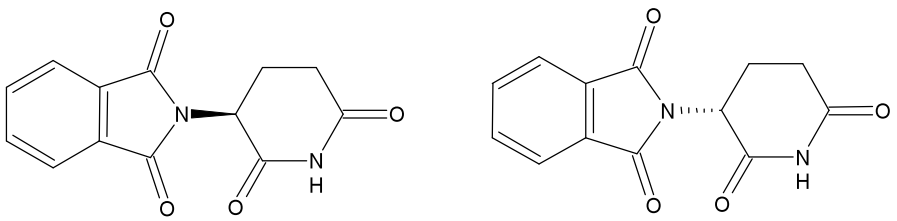
Chemoenzymatic synthesis can be described as a synthesis, using both chemical and enzymatic steps. The enzyme-catalysed reactions involving the use of pure enzymes or whole cells. A common question is '*What advantages have enzymes over the use of traditional chemical reagents*?' One important advantage nowadays is their use in *green chemistry* or *clean synthesis*. 'The challenge for chemists and others is to develop new products, processes and services that achieve the societal, economic and environmental benefits that are now required. This requires a new approach which sets out to reduce the materials and energy intensity of chemical processes and products, minimise or eliminate the dispersion of harmful chemicals in the environment, maximise the use of renewable resources and extend the durability and recyclability of products in a way which increases industrial competitiveness'.[1] For the synthetic chemist, this demands the development of a commercially viable process, where all steps are environmentally friendly. Enzymes fit this role perfectly because (i) normal enzymatic reactions are carried out in water, (ii) there are generally no

waste products produced, and (iii) as biocatalysts their productivity may be higher than any chemical catalyst and enzymes are therefore on the whole, reasonably inexpensive. A good example of the use of enzymes as an environmentally friendly option is provided by a review by *H. Danner* and *R. Braun*[2] showing how certain commodity chemicals can be produced using microorganisms to metabolise sugars, as a renewable resource, thus further avoiding the consumption of fossil fuels, normally used to produce the same commodity chemicals.

A further benefit of using enzymes in synthesis is that nature has supplied the chemist with an amazing variety of biocatalysts that may catalyse a multitude of synthetic steps. It has often been found that a single type of enzyme-catalysed reaction would require a multistep chemical synthesis, at great cost and with considerable waste production. This is evident from the ability of some enzymes to catalyse asymmetric syntheses and kinetic resolutions with a remarkable degree of chemoselectivity, regioselectivity and enantioselectivity far exceeding any chemical reagent equivalents.

1.2. The importance of chirality in chemistry.

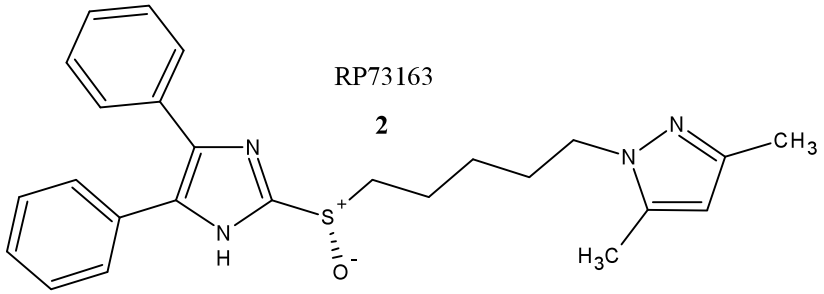
A large part of modern synthetic chemistry is devoted to chiral synthesis. This is mainly due to the needs of the pharmaceutical industry for therapeutic agents, containing chiral functionalities. In recent years there has been a growing need for single enantiomers of drugs. Perhaps the most infamous chiral drug is thalidomide **1**. This tragedy occurred in the last century, where thalidomide was marketed as a racemate, for treatment of morning sickness.



(S)-1 **Thalidomide** ***(R)-1***

Many thousands of children were born with limb deformities. It was only after this disaster that it was discovered that the different enantiomers of thalidomide **(*S*)-1** and **(*R*)-1** produced completely different biological responses. Enantiomer **(*S*)-1** was the main culprit, giving a teratogenic response while enantiomer **(*R*)-1** showed the desired sedative effects.

In organosulfur compounds, the sulfoxide group is often the sole chiral moiety. Chiral sulfoxides are becoming more frequently used as new drugs, *e.g.* the ACAT (acyl-CoA cholesterol acyltransferase) inhibitor RP73163[3] **2.** In the quest for synthetic routes to optically pure sulfoxides, biological methods are becoming more widely used. The importance of chiral sulfoxides in synthesis will be discussed later in this chapter.



RP73163

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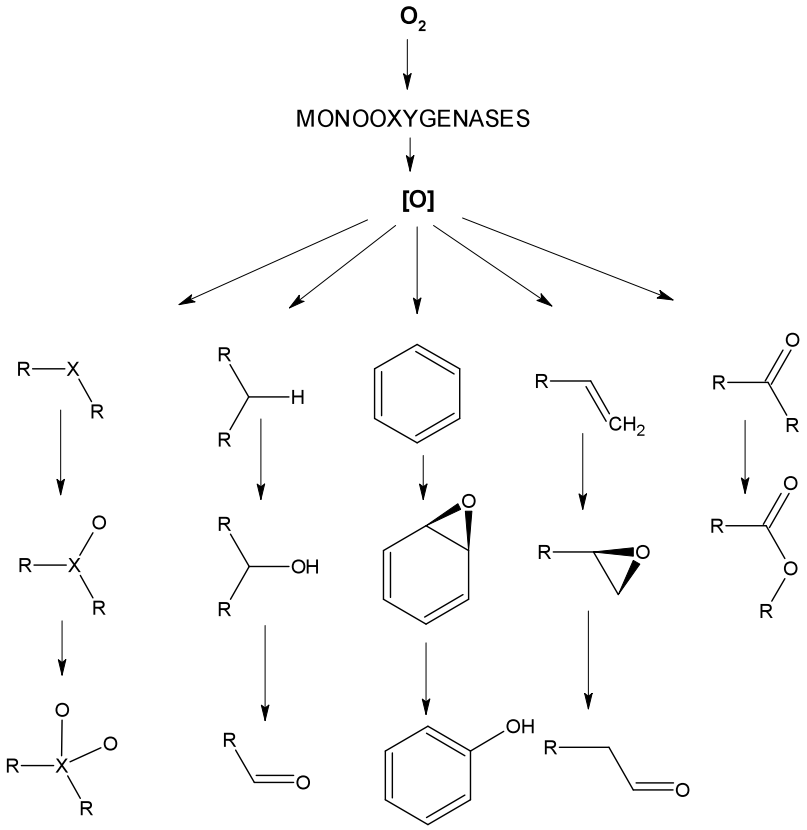
1.3. Metabolic enzymes in animals.

For centuries, and especially after the industrial revolution, humans have introduced a wide range of chemicals into the environment. These include medicines, herbicides, pesticides, industrial waste and volatile organic compounds, including mono- and poly-cyclic aromatic hydrocarbons (PAHs), which are released into our environment *via* the combustion of fossil fuels, oil spills *etc*. What happens to these compounds when they enter a human body? Are they excreted, or are they absorbed? What damage is caused by xenobiotic compounds or their metabolites?

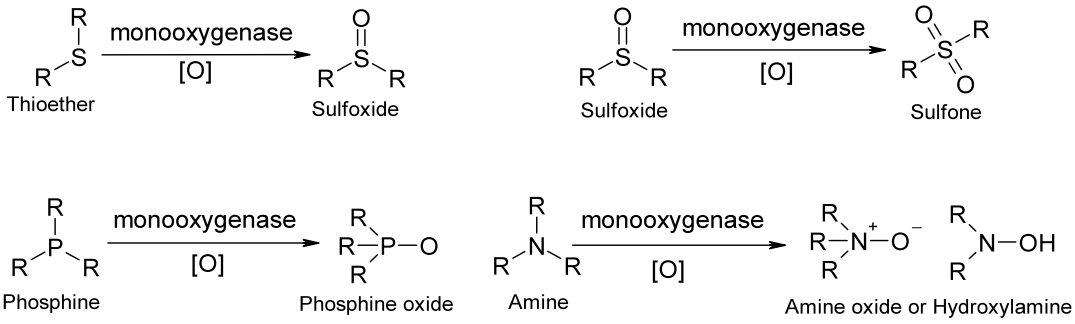
In mammals (eukaryotes), they are transformed by various enzymes until they are sufficiently polar and water soluble to be excreted. Metabolism of this type can be considered as two stages, *i.e.* Phase I and Phase II metabolism. In Phase I metabolism, many types of biotransformation can occur to break up and polarise the molecules. These include oxidations, reductions or hydrolysis reactions, many of which introduce polar groups. Other water-soluble molecules or groups, *e.g.* monosaccharides and peptides, can be used to conjugate with the phase I metabolites (Phase II metabolism).

This further increases their solubility in water and therefore their chances of excretion. In Phase I mammalian metabolism, monooxygenases (cytochrome P-450 isozymes) are the most important enzymes. The cytochrome P-450 monooxygenases (CYP-450s) are involved in oxidative metabolism and catalyse the addition of molecular oxygen to the substrate, with one of the dioxygen atoms going to the substrate and the other being reduced to water. The most common types of oxidation catalysed by monooxygenases (**Scheme 1.1.**) include heteroatom oxidation (*e.g*. sulfide, sulfoxide, phosphine and nitrogen oxidation to sulfoxides, sulfones, phosphine

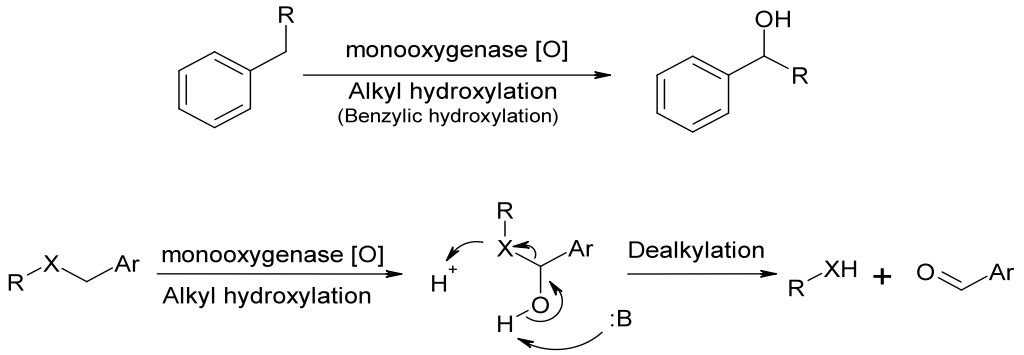


Scheme 1.1*. Common oxidations catalysed by monooxygenases.*

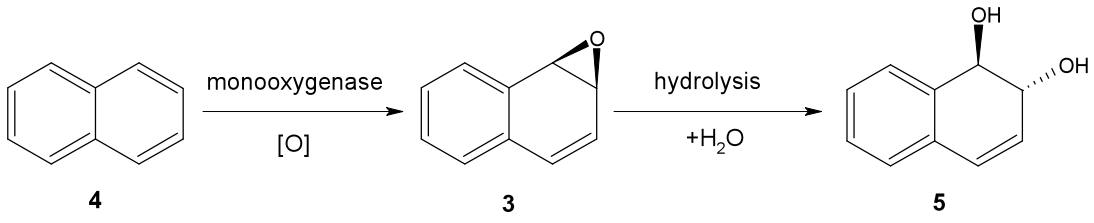
oxides, hydroxylamines and N-oxides respectively, **Scheme 1.2.**), alkyl hydroxylation, dealkylation of heteroatoms (includes heteroatom release, **Scheme 1.3.**), and Baeyer-Villiger oxidations (ketone to esters). When aromatic systems, such as PAHs, are metabolised, monooxygenases will oxidise the rings to epoxides (arene oxides) which isomerise to phenols.



Scheme 1.2. *Heteroatom oxidation catalysed by monooxygenases.*



Scheme 1.3*. Alkyl hydroxylation catalysed by monooxygenases.*



Scheme 1.4. *Arene oxide formation and hydrolysis to trans-dihydrodiol* ***5****.*

trans-Dihydrodiols are also obtained *via* the hydrolysis of unstable arene oxides (**Scheme 1.4.**). This was first demonstrated[4] by the detection of naphthalene-1,2-oxide **3** from the metabolism of naphthalene **4** followed by enzyme-catalysed

hydrolysis to yield *trans*-1,2-dihydronaphthalene-1,2-diol **5**. Arene oxides of quinoline[5] **6**, methylbenzoate[6] **7** and more recently of methyl-2-(trifluoromethyl)benzoate[7] **8** have been isolated. PAH arene oxides are known to be initial metabolites leading to diol epoxide carcinogens.

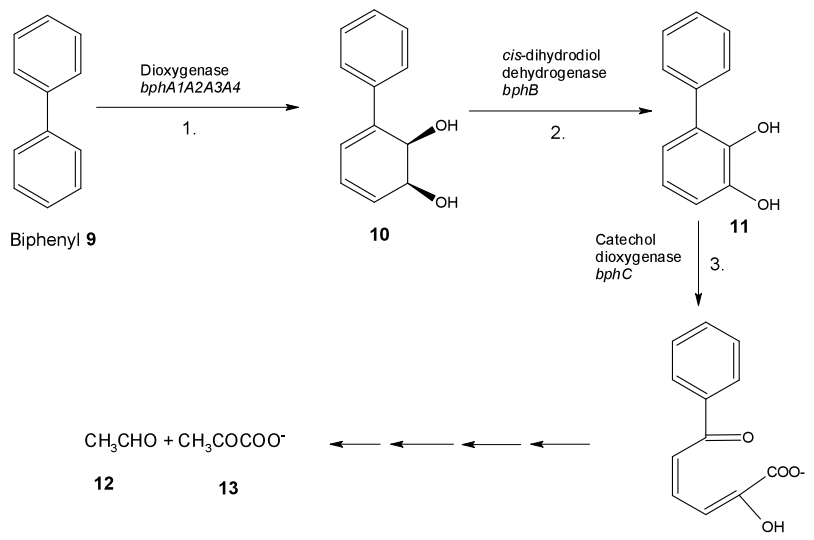
1.4. Redox enzymes in synthesis.

Applications of oxidoreductase enzymes are extremely important in metabolism. Within this category, monooxygenases, dioxygenases, dehydrogenases and reductases have all been used as biocatalysts in chemoenzymatic synthesis. In the context of this thesis particular emphasis will be placed upon dioxygenases.

1.4.1. Dioxygenases in nature.

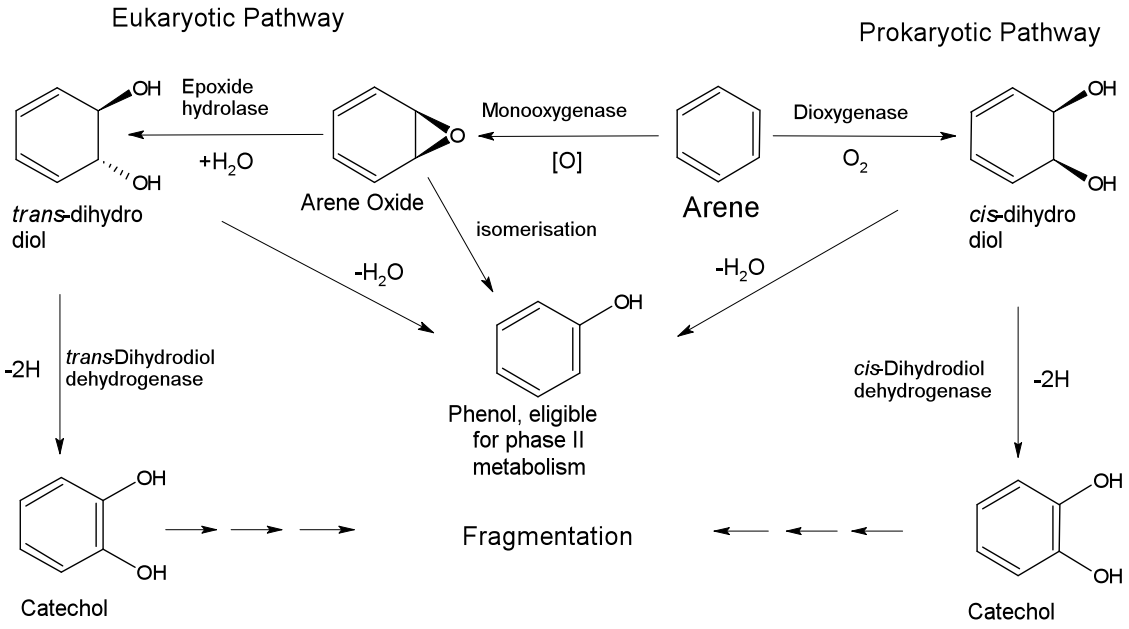
Due to the harmful biological effects of PAHs, found in our environment, there has been much interest in studying their metabolism. As cytochrome P-450 systems are among the most important in animal metabolism, early studies focussed around these. Recently the study of metabolism by other oxidative enzymes has become more common.

One of the primary processes for the removal of contaminating PAHs from our environment is thought to be *via* microbial degradation.[8] The biodegradation of a wide range of xenbiotics by microorganisms, has been studied[9] and it has been determined that dioxygenases play a major role in the bacterial catabolism of aromatics. Several biocatalytic steps, including the initial oxidation step of PAHs, involve dioxygenases. Thus, in the degradation of biphenyl **9** (see **Scheme 1.5.**) by the wild type strain *Sphingomonas yanoikuyae* B1, as described by Zylstra and Kim,[10] the initial step (step 1) involves dioxygenase attack, giving a *cis*-dihydrodiol **10**. Dehydrogenation to a catechol **11** (step 2), followed by further dioxygenase-catalysed attack (step 3) giving ring cleavage. Subsequent catabolic steps, also involving dioxygenases are carried out on the fragments, ultimately leading to acetaldehyde **12** and pyruvate **13** which then become involved in normal metabolic pathways in the bacterium. The initial steps in the metabolism of arenes are similar in some respect to the eukaryotic catabolic metabolism of aromatics. (**Scheme 1.6.**).



***Scheme 1.5**. The bacterial catabolism of biphenyl* **9**.

Eukaryotic metabolism, involve arene oxide formation as the initial step followed by hydrolysis to yield a *trans*-dihydrodiol. Both arene oxides and *trans*-dihydrodiols can form phenols. The *trans*-dihydrodiols are also converted to catechols prior to further degradation. The initial step in prokaryotic metabolism involves *cis*-dihydrodiol formation, which may be again converted to phenols or catechols.



***Scheme 1.6**. Prokaryotic and eukaryotic arene metabolism*.

1.4.2. Catalytic mechanism of dioxygenases.

Dioxygenases are known to be multicomponent enzymes, comprising several proteins involved in the transport of electrons to the active site, binding of substrate and actual biocatalytic oxygenation of the substrate at the active site.[11] In the case of naphthalene dioxygenase (NDO) from *Pseudomonas sp*. NCIMB 9816-4, an X-ray crystal structure has been obtained[12] (**Fig 1.1.**). The active part of the enzyme contains two domains. One containing an $[Fe_2S_2]$ cluster, called a Rieske centre, and the active site that is a mononuclear co-ordinated iron (**Fig 1.2.**). In the Rieske centre, one iron atom is co-ordinated by Cys 81 and Cys 101 residues, while the second iron atom is co-ordinated by His 83 and His 104 residues. The active site mononuclear iron atom is co-ordinated by His 208, His 213, bidentate Asp 362 residues and water. Electrons, originating from NADH are passed from the Rieske centre, through Asp 205 residue which is hydrogen bonded to both His 104 of the Rieske centre and His 208 of the active site, where it has been determined, from $^{18}O_2$ labelling studies, that both atoms of molecular oxygen are incorporated into the substrate.[13,14] The exact mechanism of the addition of dioxygen to the substrate is unknown. The mechanism was originally thought to involve a dioxetane intermediate[15,16] (**Scheme 1.7.**), but there is no evidence to support this other than the incorporation of both atoms of O_2 from the $^{18}O_2$ studies. It is also now considered that the four membered dioxetane ring is unlikely to exist. It would be too unstable due to the ring strain involved, and also the loss of aromaticity of the arene substrate. There is now X-ray crystallographic evidence available for the existence of an acyclic peroxide intermediate.[14] A refined crystal structure of naphthalene dioxygenase (NDO), obtained from *Pseudomonas sp*. NCIMB 9816-4, grown in the presence of indole **14** derived from the metabolism of tryphtophan **15**, showed the presence of an indole substrate bound through a peroxide bond to an iron atom in the active site (**Fig 1.3.** & **Scheme 1.8.**).

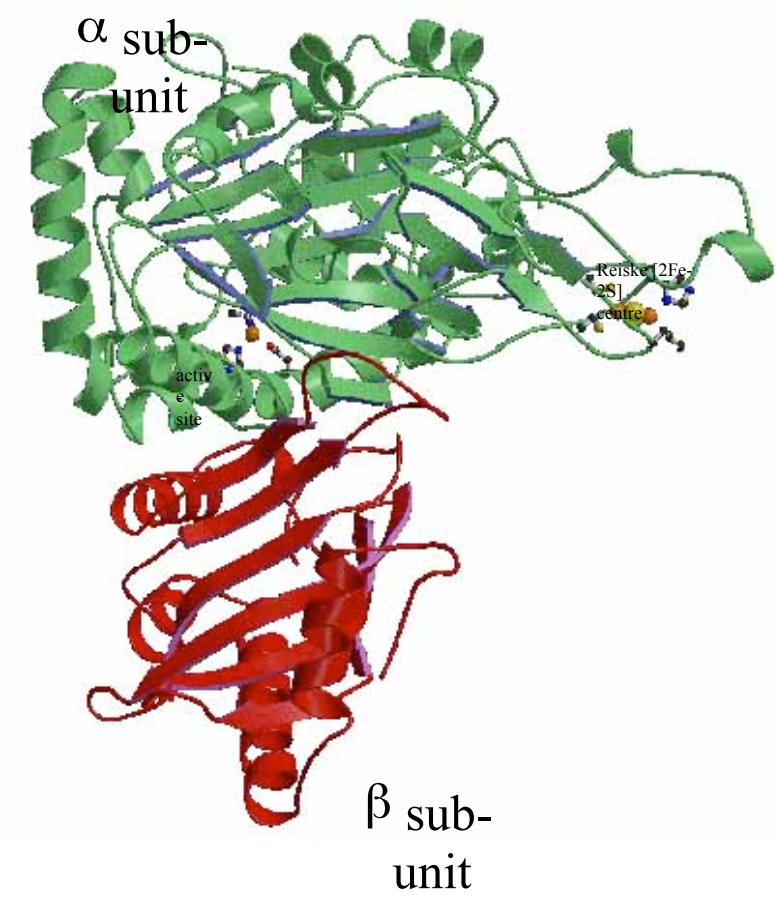


***Fig 1.1**. Crystal structure showing active site and Rieske centre of NDO.*

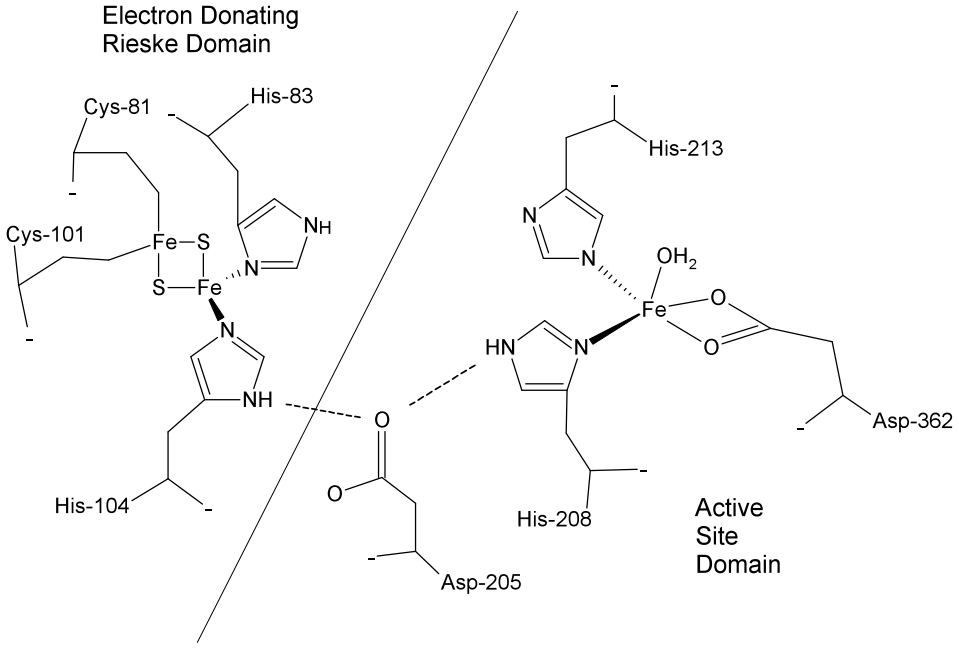
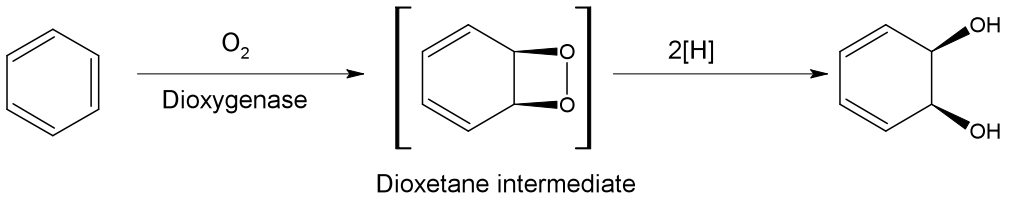
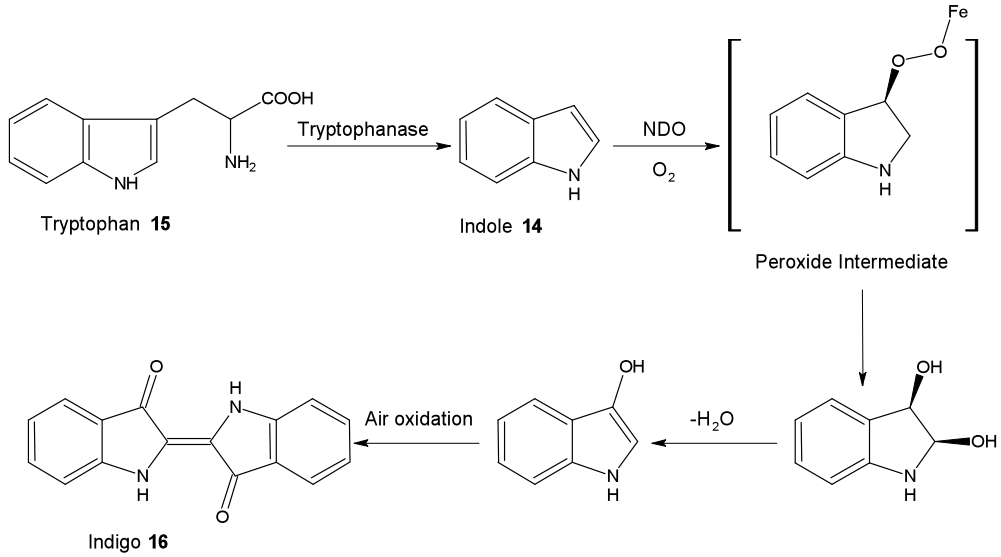


***Fig 1.2.** Electron transport in NDO.*



Scheme 1.7. *Proposed formation of dioxetane intermediate.*



Scheme 1.8. *Formation of acyclic peroxide intermediate during indigo* ***16*** *production.*

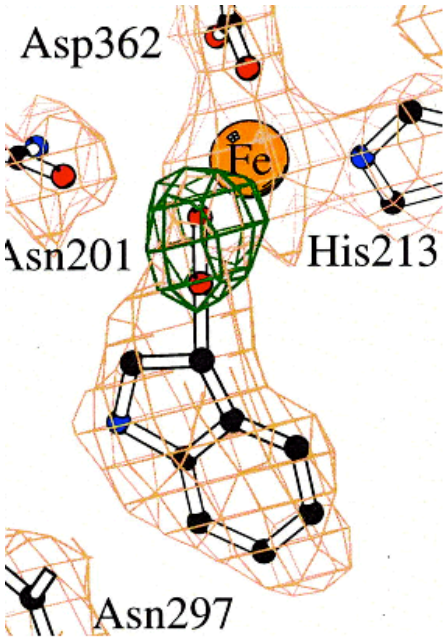
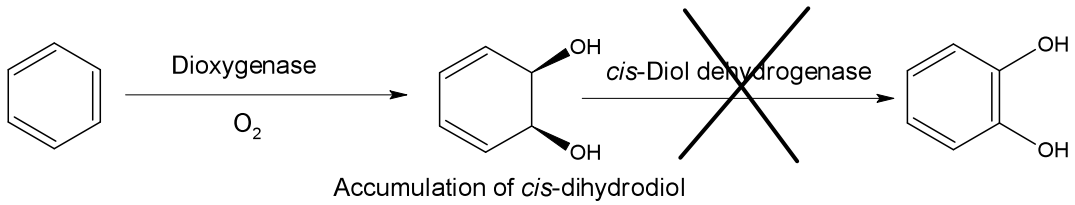


Fig 1.3. *X-ray structure showing a peroxide intermediate.*

1.4.3. Types of dioxygenase enzymes.

To date there have been as many as 40 different dioxygenases identified by protein purification and sequence analysis.[17,18] Different strains of dioxygenase-containing micro-organisms have been grown using single organic compounds as the sole carbon and energy sources. The most common method used for distinguishing different dioxygenases is to name them after the carbon source. In this study we have been using TDO (**T**oluene **D**i**O**xygenase) grown on toluene, NDO (**N**aphthalene **D**i**O**xygenase) grown on naphthalene and a BPDO (**Bi**-**P**henyl **D**i**O**xygenase) grown on biphenyl as the sole carbon and energy source. TDO was initially purified, characterised and classified by Gibson.[13,19,20] NDO was first isolated from a *Pseudomonas sp*. NCIMB 9816.[21] Its X-ray structure was however determined from NDO isolated from strain NCIMB 9816-4[12] (**Fig 1.1**). BPDO was isolated by Gibson in 1973[22] from a *Beijerinckia* species (B1) and this strain has recently been reclassified as *Sphingomonas yanoikuyae* B1.[23] For the biotransformations described herein *Pseudomonas putida* UV4 (TDO), *Pseudomonas sp*. 9816-11 (NDO) and *Sphingomonas yanoikuyae* B8/36 (BPDO) have been used. These strains are mutated forms of the wild type strains, where the mutation alters the genome of the micro-organism in order to prevent the expression of the *cis*-diol dehydrogenase, in the catabolic sequence, thus giving an accumulation of *cis*-dihydrodiol bioproduct (**Scheme 1.9.**). The NDO and BPDO strains are single mutants which need to be fed on a growth medium but also require an inducer in the form of a small organic molecule such as salicylic acid or xylene, in order for the dioxygenase to be expressed. This inducer molecule binds to a repressor protein, causing it to stimulate the transcription and translation of the genetic code for the dioxygenase enzyme. The UV4 strain, a commercial strain, is a doubly mutated strain. The initial mutation caused the prevention of expression of *cis*- diol dehydrogenase.

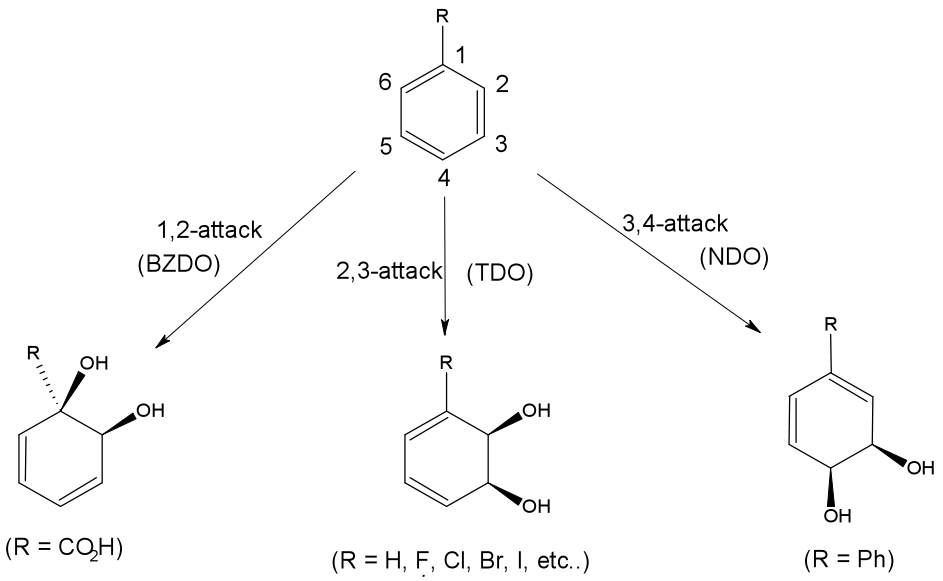


Scheme 1.9. *Incomplete metabolism giving accumulation of a cis-dihydrodiol.*

The second mutation promotes the continuous expression of the dioxygenase by altering the bacterial genetic code for the repressor protein so as to stimulate the expression of the dioxygenase permanently, without the need for an inducer. Therefore the UV4 strain requires only a growth medium and the substrate for a biotransformation. The best substrates for these dioxygenases are the carbon sources upon which the wild type strains are grown. The enzymes are not restricted to metabolising these substrates alone. Many arene substrates with similar shape and size to the initial carbon source can be metabolised by differing routes. This is demonstrated by the fact that dioxygenases are capable of catalysing benzylic hydroxylation, heteroatom oxidation (including chiral sulfoxidation) as well as producing many types of aromatic dihydroxylation products from the biotransformation of a range of substrates.

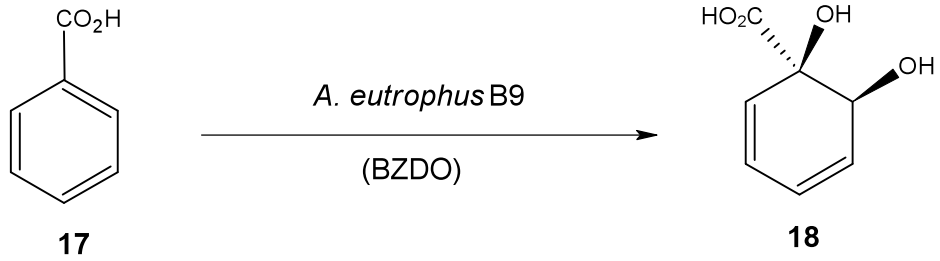
1.4.4. Common dioxygenase-catalysed reactions.

Over 300 *cis*-diols have been isolated as metabolites from arene substrates. These range from monosubstituted aromatics to pentacyclic PAHs, as summarised in two recent reviews.[24,25] The majority of substrates studied are monosubstituted benzenes which on metabolism with *P. putida* UV4 (containing TDO as biocatalyst) yield *cis*-diols after dihydroxylation- exclusively across the 2,3-bond (see **Scheme 1.10.**).



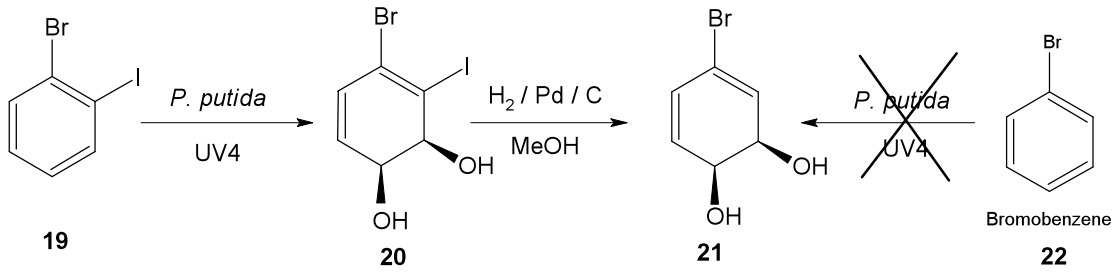
Scheme 1.10. *Regiospecific attack by dioxygenases on monosubstituted benzenes*.

There is only one example of *cis*-dihydroxylation occurring across the 1,2- bond[26,27] *i.e.* the metabolism of benzoic acid **17** by *Alcaligenes eutrophus* B9, containing BZDO (**B**en**Z**oate **D**i**O**xygenase), to yield *cis*-cyclohexadiene-1,2-diol-1-carboxylic acid **18** (**Scheme 1.11.**). It has only recently been reported that a 3,4-diol is formed during the metabolism of biphenyl with site directed mutants containing NDO.[28] 3,4-*cis*-Dihydrodiols have also been synthesised by chemoenzymatic methods from the corresponding 2,3-*cis*-diol bioproducts.[29]



Scheme 1.11. *Formation of a 1,2-dihydrodiol* ***18*** *by A. eutrophus B9.*

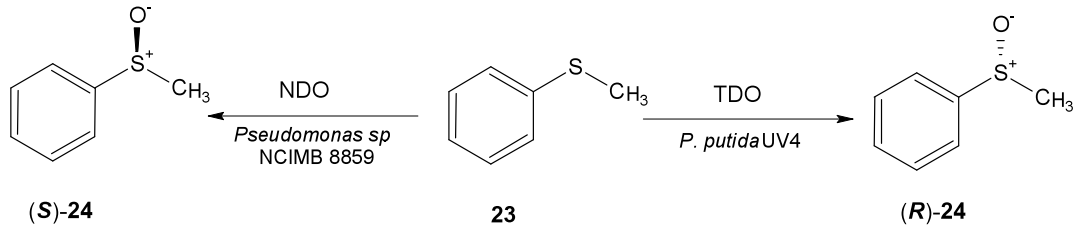
1-Bromo-2-iodobenzene **19** was thus metabolised by *P. putida* UV4 giving (1*S*,2*S*)-4-bromo-3-iodocyclohexa-3,5-diene-1,2-diol **20** in > 98% ee. This was hydrogenolysed using a palladium catalyst to give (1*S*,2*R*)-4-bromocyclohexa-3,5-diene-1,2-diol **21** in 65% yield (**Scheme 1.12.**).



Scheme 1.12. *Chemoenzymatic synthesis of the 3,4-cis-diol* **21**

In addition to aromatic dihydroxylation, dioxygenases have the ability to catalyse other reactions such as benzylic hydroxylations, and heteroatom oxidations. A good example of heteroatom oxidation by dioxygenases is the production of a range of chiral sulfoxides with high ee values from the corresponding sulfides. In several cases enantiocomplementarity was observed with different dioxygenases., *e.g.*

sulfoxidation of thioanisole **23** with NDO and TDO from *Pseudomonas sp*. NCIMB 8859 and *Pseudomonas putida* UV4 respectively[30] (**Scheme 1.13.**). From use of the NDO enzyme the (*S)* enantiomer of methyl phenyl sulfoxide **24** is formed with an ee of 91% and a 33% yield. However, with the TDO the (*R*) enantiomer is formed exclusively in 90% yield.



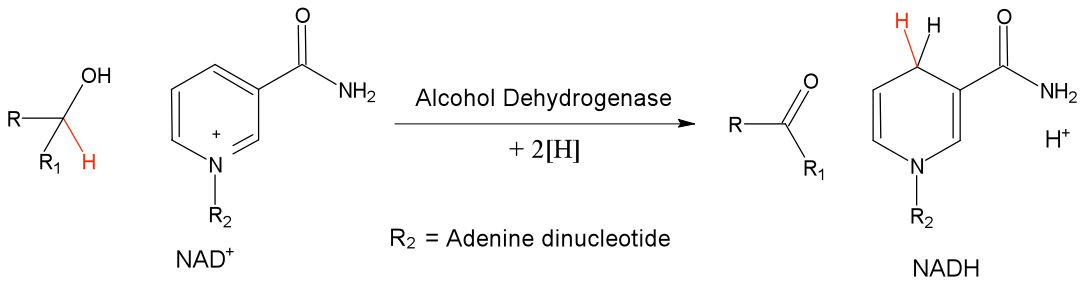
Scheme 1.13. *NDO- and TDO- catalysed sulfoxidation of thioanisole* ***23***.

1.4.5. Dehydrogenases and reductases.

Dehydrogenases and reductases also play an important role in metabolism. They catalyse various reactions such as hydrogenation and dehydrogenation of multiple bonds, carbon-heteroatom bond cleavage (*e.g*. dehalogenation) and heteroatom deoxygenation (*e.g*. sulfoxide reduction to sulfide).

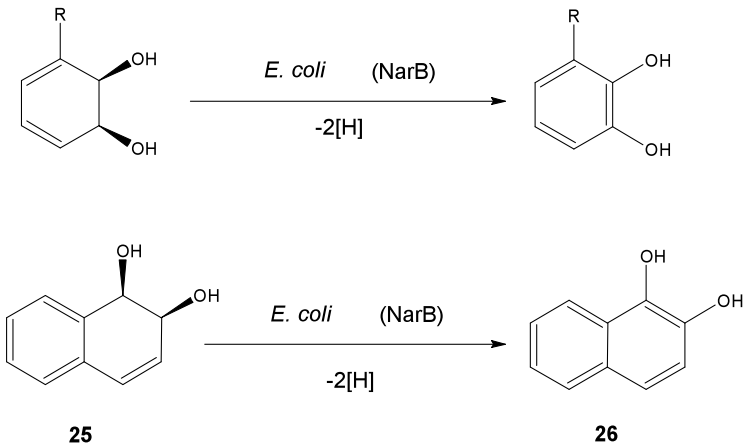
1.4.5.1. *cis*-Dihydrodiol dehydrogenases.

Dehydrogenase enzymes such as alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase and soluble malate dehydrogenase are important in metabolism. These enzymes catalyse the oxidations of the appropriate alcohol to acetaldehyde, glyceraldehyde-3-phosphate to glyceric acid-1,3-biphosphate, lactic acid to pyruvic acid and malic acid to oxaloacetic acid respectively. The best known alcohol dehydrogenase catalyses the oxidation of ethanol to acetaldehyde. In common with many of the well known alcohol dehydrogenases, *e.g.* those isolated from horse liver or yeast, it contains zinc at the active site. These enzymes catalyse the loss of 2 e^- and 2 H^+ from an alcohol, involving a co-factor, usually $NAD,^+$ and releasing an aldehyde or ketone plus $NADH + H^+$ (**Scheme 1.14.**).

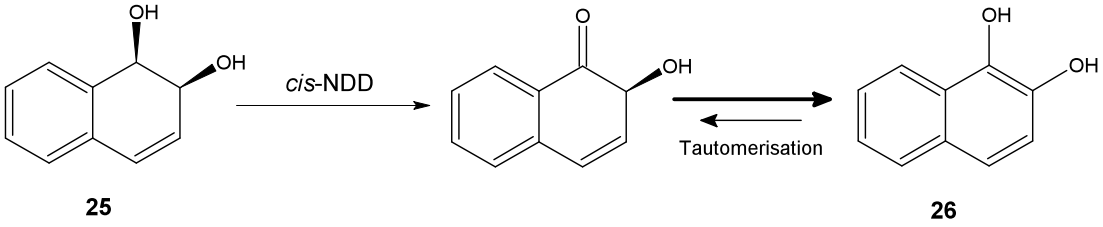


Scheme 1.14. *Enzyme-catalysed oxidation of an alcohol.*

An *E. coli* strain, which expresses the naphthalene *cis*-dihydrodiol dehydrogenase gene (narB) from *Rhodococcus sp*. NCIMB 112038 strain, has been used in the work presented in this thesis.[31] As shown in **Schemes 1.5**, **1.6** and **1.9,** *cis*-dihydrodiol dehydrogenases are involved in prokaryotic arene catabolism. The new *Escherichia coli* strain (narB) has the ability to convert several *cis*-dihydrodiols into catechols (see **Scheme 1.15.**). Previously Jeffrey *et al*[32] had discovered that **n**aphthalene *cis*-**d**ihydrodiol **d**ehydrogenase (*cis*-NDD) *from P. putida* 9816 oxidised the dihydrodiol **25** to the corresponding catechol **26** *via* a non-concerted mechanism, similar to the alcohol dehydrogenases, **Scheme 1.14.** This involves the oxidation of one of the hydroxyl groups to give an unstable ketoalcohol intermediate that spontaneously tautomerises to the catechol **26** (**Scheme 1.16.**). Preliminary work has shown that the 'narB' enzyme has the ability to dehydrogenate a range of monocyclic and polycyclic *cis*-dihydrodiols, such as naphthalene- *cis*-1,2-dihydrodiol **25** to 1,2-dihydroxynaphthalene **26**. *trans*-Dihydrodiols however are not dehydrogenatated by this enzyme.



Scheme 1.15*. Enzymatic dehydrogenation of cis-dihydrodiols.*

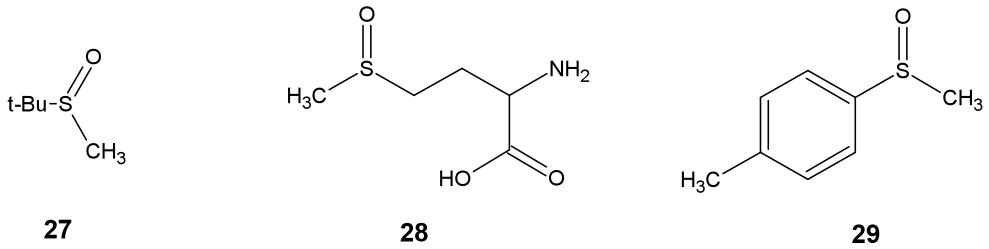


Scheme 1.16. *Non-concerted formation of a catechol* ***26*** *by cis-NDD.*

1.4.5.2. DMSO Reductases.

Bacterial DMSO reductases found in nature play an important part in the regulation of dimethyl sulfoxide (DMSO) and dimethyl sulfide (DMS) in the global sulfur cycle[33] (**Fig 1.4.**). Thus DMSO, formed mainly from photooxidation of volatile DMS in the atmosphere, is converted to DMS by means of a deoxygenation reaction, catalysed by DMSO reductases found in ocean-dwelling bacteria.

This deoxygenation reaction has to date been applied to a small range of dialkyl and alkyl aryl sulfoxides, by Hanlon *et al.*[34] t-Butylmethyl sulfoxide **27**, methionine sulfoxide **28** and the alkyl aryl sulfoxide methyl-p-tolyl sulfoxide **29**, were reduced by whole-cell organisms and purified enzymes. The potential importance of reductase enzymes to the synthetic chemist lies in their ability to kinetically resolve a sulfoxide racemate. Some DMSO reductases have even been found to catalyse the deoxygenation of trimethylamine N-oxide.



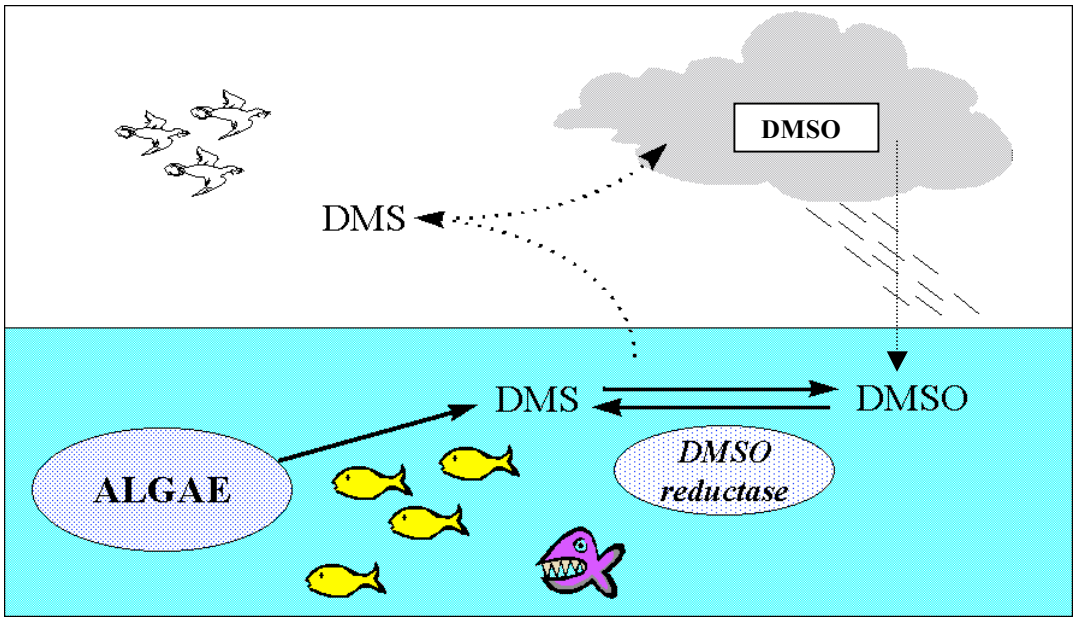
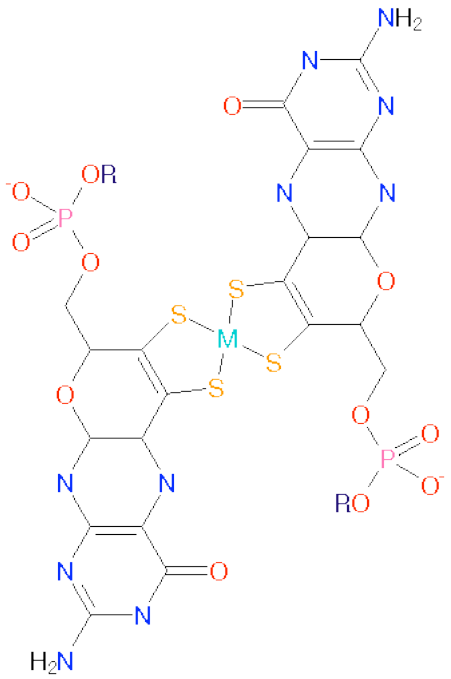


***Fig1.4**. The global sulfur cycle.*

The redox center of the enzyme, where the actual deoxygenation occurs is part of a cofactor containing a molybdenum atom that is co-ordinated by two pterin molecules (molybdopterin cofactor, **Fig 1.5.**). It is coordinated to the active site of the enzyme by an amino acid residue *e.g.* a serine residue, as observed in the X-ray crystal structure for DMSO reductase (**Fig 1.6.**) obtained from *Rhodobacter capsulatus* by McAlpine *et al*.[35] Crystallography studies done on DMSO reductases from other strains of bacteria show similar structures.[36-38] At the active site the molybdenum exists in two oxidative states. The reductive process begins with binding of DMSO to the reduced Mo^{IV} which is oxidised to Mo^{VI} releasing DMS and leaving the extra O + 2 e^- atom co-ordinated to the metal centre. The Mo^{VI} then receives 2 e^- from an electron donor (specific cytochrome, differing for each enzyme), allowing the regeneration of the Mo^{IV} and releasing an oxygen atom in the form of H_2O simultaneously. This then allows the binding of another DMSO and repetition of the cycle.

In this study DMSO reductases from intact cells of *Rhodobacter capsulatus*, *Escherichia coli*, *Proteus Vulgaris*, have been evaluated. In particular a new bacterium, recently isolated from the North Sea, *Citrobacter braakwi*, as part of a collaborative study with the Department of Biological Sciences at the University of Warwick.



Metal·molybdopterin cofactor
(M = Mo;R = AMP, GMP, HMP)

Fig 1.5. *Molybdopterin cofactor*.

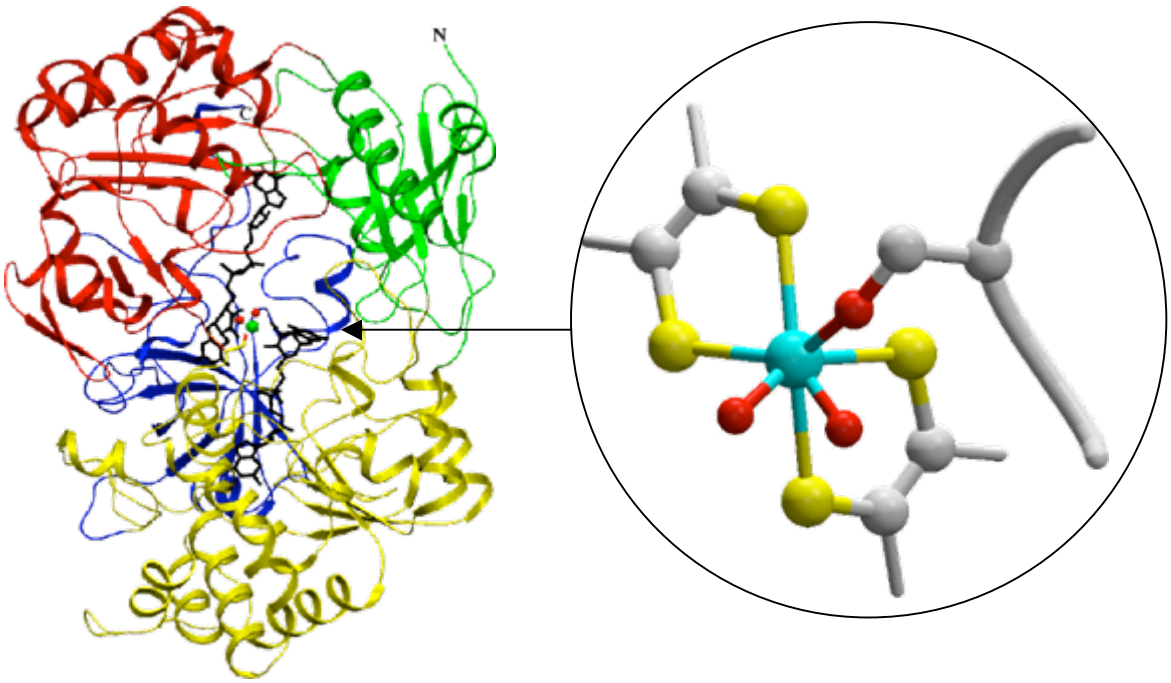


Fig 1.6. *Crystal structure for DMSO reductase from Rhodobacter capsulatus*.

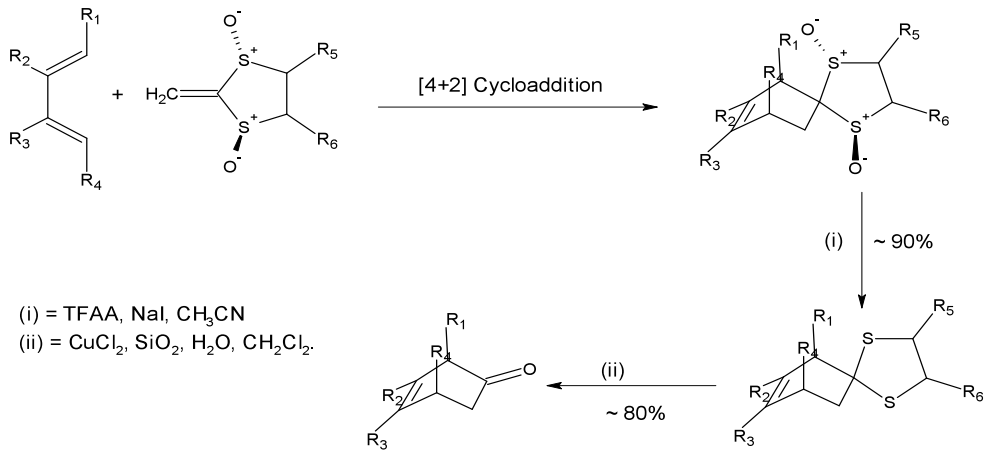
1.5. Applications of chiral sulfoxides, *cis*-dihydrodiols and catechols in synthesis.

As this research programme has been directed towards using enzymes to create chirality in organosulfur compounds, and applications of the latter, a summary of potential uses for chiral sulfoxides and other types of dioxygenase-derived bioproducts from these biotransformations is provided.

1.5.1. Applications of chiral sulfoxides.

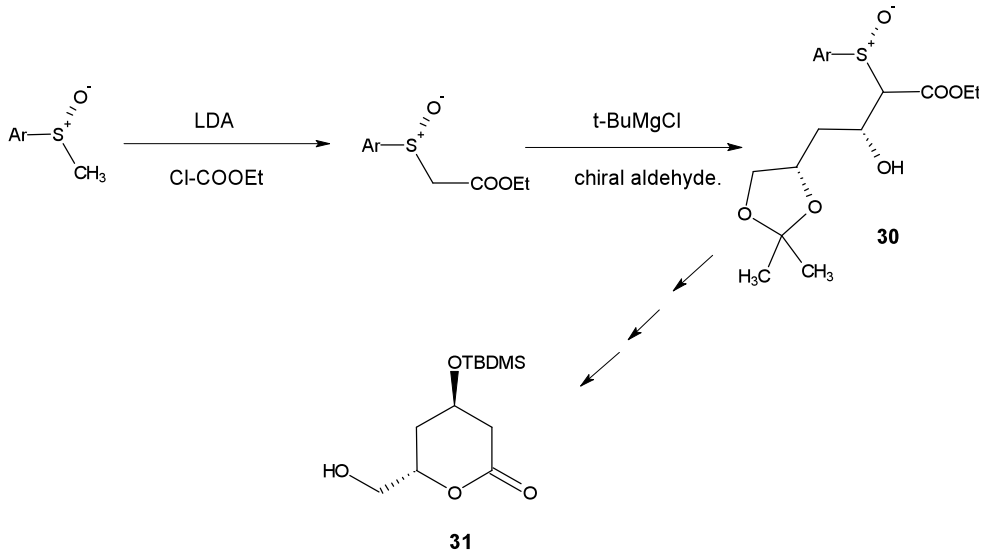
As suggested earlier in the chapter, the chiral sulfoxide functionality can be found in many agrochemical and pharmaceutical compounds and improved, or alternative, synthetic methods are constantly being sought.

Chiral sulfoxides themselves are very useful in synthesis and will facilitate such reactions as 1) carbanion formation with alkyl sulfoxides, 2) dienophile activation with vinyl sulfoxides and 3) pseudo-Michael additions. The sulfoxide is also easily converted to other functional groups. The presence of a chiral sulfoxide often provides strong stereodirecting power. This was demonstrated by the work carried out by Aggarwal *et al*[39] where chiral vinyl sulfoxides were used as dienophiles in Diels Alder cycloadditions using a range of dienes. The products showed high diastereomeric excess values (> 94% de) and were subsequently converted to ketones by reduction and cleavage of the organosulfur portion (**Scheme 1.17.**). This provides a useful demonstration of the application of sulfoxides as chiral auxilaries in synthesis



Scheme 1.17. *Synthesis of chiral ketones.*

Sulfoxides are also becoming more widely used as chiral ligands in organometallic reactions, such as diethylzinc-assisted alkylation reactions of aromatic aldehydes. Another example is the synthesis of a synthon **31** for hypocholestemic agents related to mevinic acid, from an optically pure sulfoxide.[40] An α-carbon on a single enantiomer of alkyl aryl sulfoxide was deprotonated in two successive steps to create a single diastereoisomer **30** containing new chiral centres. Further steps, including cleavage of the sulfoxide, were carried out to give the lactone **31** with 2 new chiral centres (**Scheme 1.18**.).

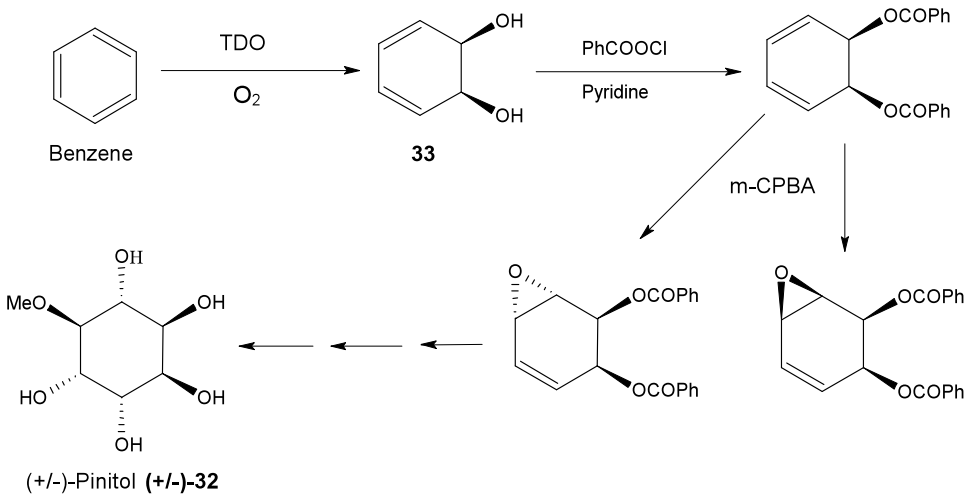


Scheme 1.18. *Use of chiral sulfoxide carbanion in synthesis*.

1.5.2. Applications of *cis*-dihydrodiols.

The use of *cis*-dihydrodiols in the synthesis of natural products has become more common over the last decade, and several reviews have been published on the topic.[41-45] Industrial applications of *cis*-dihydrodiols have also been explored, *e.g.* production of indigo dye.

The first reported biologically important molecule synthesised from a *cis*-dihydrodiol was racemic pinitol **(+/-)-32**.[46] Pinitol **32** was obtained from *cis*-cyclohexa-3,5-diene-1,2-diol **33**, the dioxygenase-catalysed oxidation product of benzene (**Scheme 1.19.**).



***Scheme 1.19**. Synthesis of racemic pinitol **32**.*

Hudlicky *et al*[47] synthesised pinitol **32** from *cis*-(1*S*,2*S*)-bromo-1,2-dihydrobenzene-1,2-diol **34** but in enantiopure form. The *cis*-dihydrodiol metabolites from monosubstututed benzenes can be applied to many types of reaction, including transfer of chirality to new exocyclic centres. They can undergo reactions such as epoxidation of double bonds, cycloadditions, selective hydrogenation of double bonds, hydrogenolysis of C-halogen bonds, protection/deprotection of diol functionality, osmylation of double bonds, reductive/oxidative ozonolysis of C=C bonds, carbene addition, formation of organometallic complexes and substitution of halogen atom (**Fig 1.7.**).

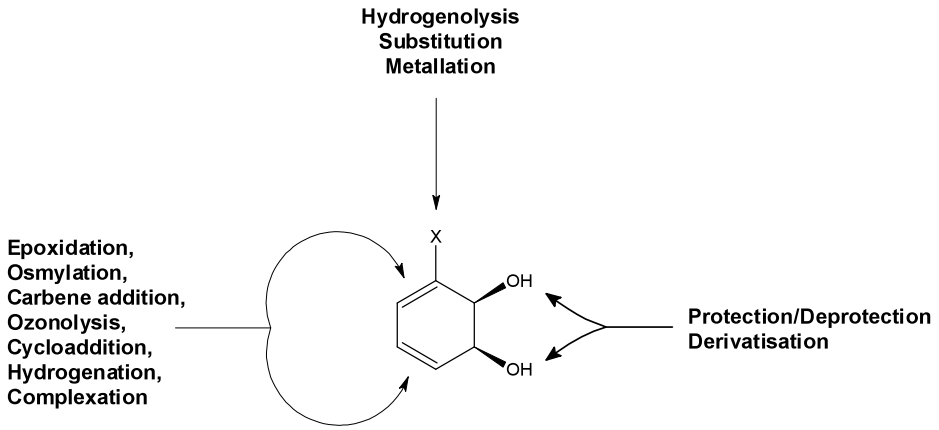
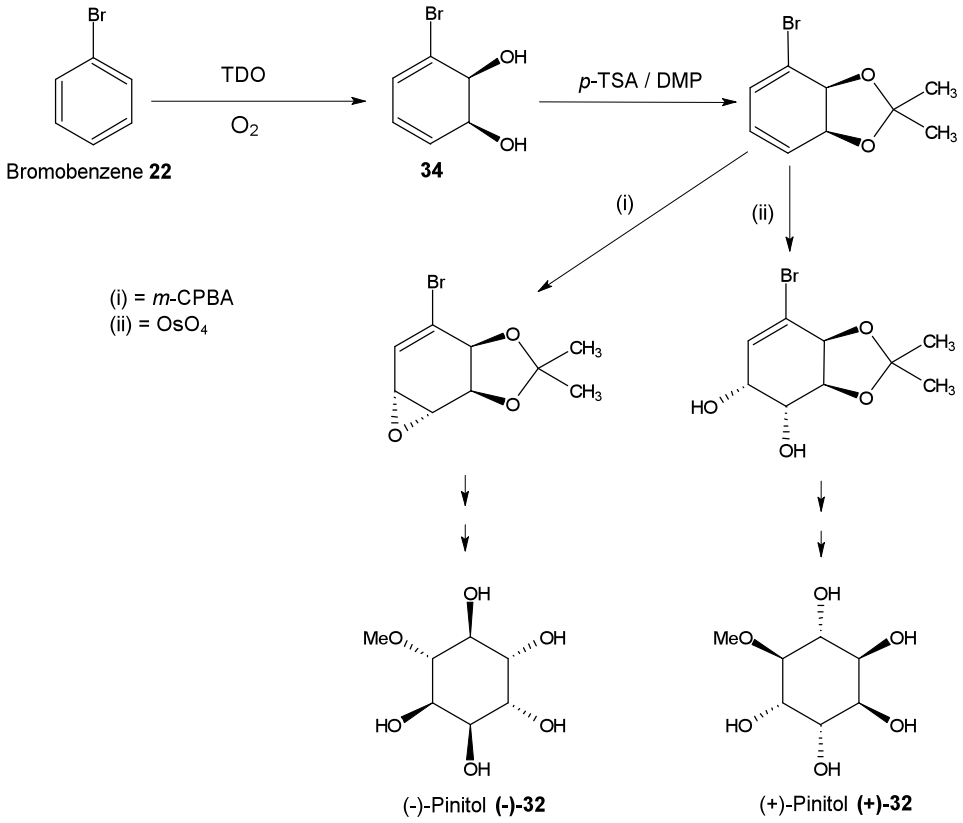


***Fig 1.7**. Reactions of cis-dihydrodiols.*



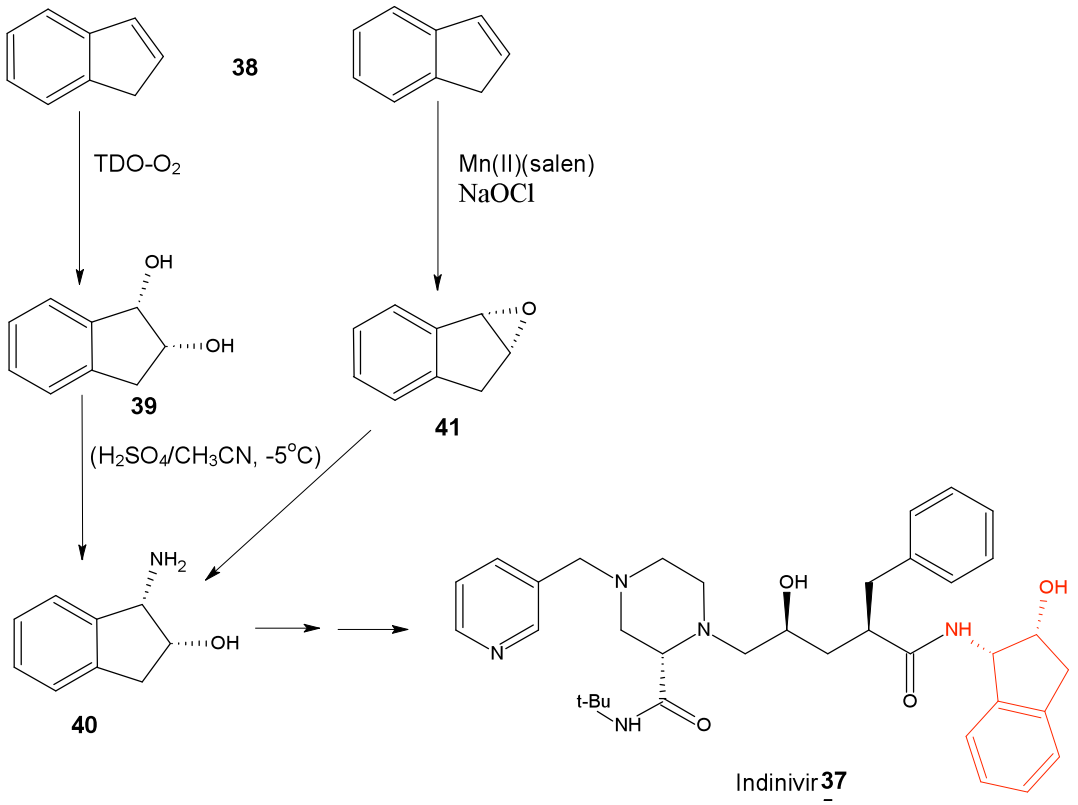
***Scheme 1.20**. Synthesis of pinitol **32** enantiomers.*

These reactions have allowed the synthesis of many natural products, natural product analogues, or compounds of pharmacological and biological importance. Pinitols, inositiols,[48-51] conduritols[41,52-54] and their analogues, *e.g.* L-*chiro*-inositol **35** and (-)-conduritol E **36**, are some typical examples which have been synthesised from enantiopure *cis*-diols.

1.5.2.1. Potential applications of *cis*-dihydrodiols in industry.

Some *cis*-dihydrodiols are now commercially available in multigram quantities. Inositols synthesised from *cis*-dihydrodiols are also commerically available on a medium scale for natural product research. On a larger scale indigo dye **16** has been produced by Genencor International from the NDO-catalysed oxidation of indole **14** as shown in Scheme 1.8. In the pharmaceutical industry, Indinivir (crixivan) **37**, an important HIV protease inhibitor developed by Merck, has been synthesised,[55] by a method involving the dioxygenase-catalysed *cis*-dihydroxylation of indene[56] **38** to give (1*S*,2*R*)-dihydroxyindan **39**. This is converted, by the Ritter reaction, into the

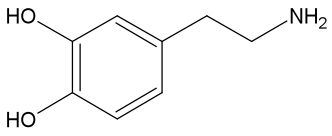
amino alcohol (1*S*,2*R*)-1-amino-2-hydroxyindan **40**, and is then used to construct indinavir **37**, containing the aminoalcohol moiety. The alternative to this process is the Jacobsen epoxidation of indene **38** to give the indene oxide **41** followed by the Ritter reaction to give the amino alcohol **40**. The advantage of the biological method is that the Jacobsen epoxidation[57] only gives *ca*: 86% ee whereas the biological method produces the enantiopure diol **39** (**Scheme 1.21.**).



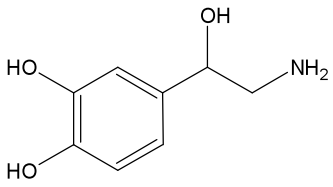
Scheme 1.21. *Industrial synthesis of Indinivir 37.*

1.5.3. Catechols.

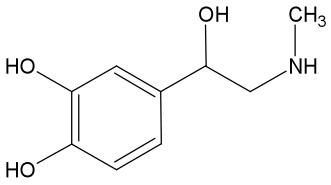
Catechols and substituted catechols are components of many natural products found in the environment and biologically active molecules in animals. Neurotransmitters such as dopamine **42**, norepinephrine (noradrenaline) **43** and epinephrine (adrenaline) **44** all contain catechol functionalities.



Dopamine **42**

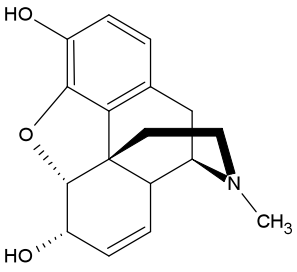


Norepinephrine **43**

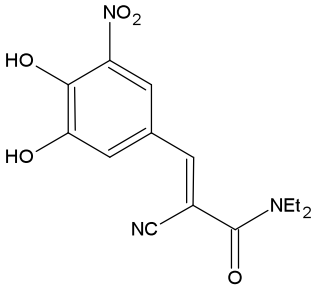


Epinephrine **44**

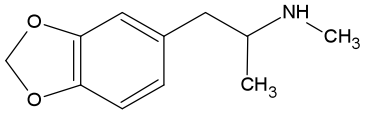
Drugs such as the narcotic painkiller morphine **45** or Entacapone **46**, marketed by Novartis for the treatment of Parkinson's disease, also contain catechol moieties. Drugs of abuse such as methylenedioxy methamphetamine (MDMA) **47** also known as 'Ecstasy', often contain catechols.



Morphine **45**

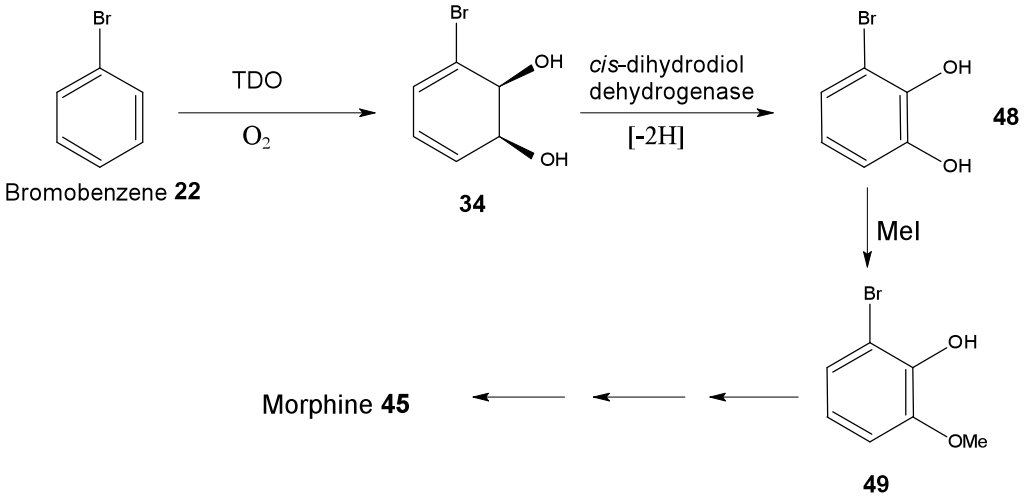


Entacapone **46**



MDMA **47**

A catechol precursor has also been used,[58,59] in approaches to the syntheses of morphine **45**. In this approach, catechol **48**, and its monomethyl ether derivative bromoguiacol **49**, were used as synthetic precursors. Catechol **48** has been derived from bromobenzene **22** using a tandem biotransformation, *i.e.* two enzymes in the one pot.[60] This process involves (i) a dioxygenase-catalysed oxidation of bromobenzene **22** to give the *cis*-diol **34**, (ii) dehydrogenation with a *cis*-dihydrodiol dehydrogenase enzyme to yield 2,3-dihydroxybromobenzene **48**, followed by mono-methylation to give the substituted catechol **49** (**Scheme 1.22.**).



Scheme 1.22. *Synthesis of morphine **45**.*

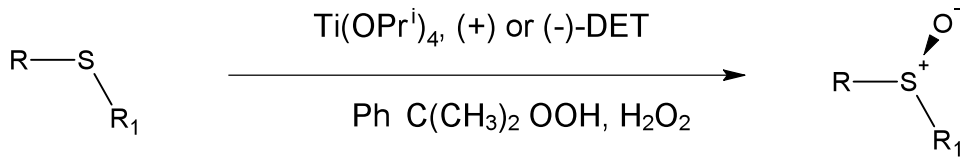
1.6. Synthetic approaches to chiral sulfoxides.

Non-racemic chiral compounds can be obtained by methods involving asymmetric synthesis or various resolution techniques. The asymmetric synthesis route involves preferential attack by a reagent on a prochiral face, atom or group to give an excess of a single enantiomer. The chirality may be induced by the chiral reagent, or by an existing chiral centre on the substrate, forming diastereoisomers in varying proportions, which may be separable by chromatographic techniques. The kinetic resolution method involves the chemical transformation of enantiomers of a racemic mixture at differing rates, leaving an excess of one enantiomer. This will be discussed in greater detail in Chapter 2.

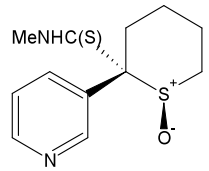
There are several methods in the literature for the synthesis of chiral sulfoxides involving asymmetric synthesis and kinetic resolution. The best known methods are those developed by Kagan[61,62] and Andersen.[63]

(i) The 'Kagan method' is an extension of the 'Sharpless method' for epoxidation of allylic alcohols. It provides a good general method for asymmetric synthesis of chiral sulfoxides from prochiral sulfides. Like the Sharpless method, it involves use of $Ti(OPr^i)_4$, tert. butyl hydroperoxide and diethyltartrate. The main difference involves addition of 1 mol of H_2O to co-ordinate to the Ti atom, replacing the co-ordinating hydroxyl in allylic alcohols. The best ee values were obtained using cumene hydroperoxide, instead of t-butyl hydroperoxide[64] (**Scheme 1.23.**). This method has

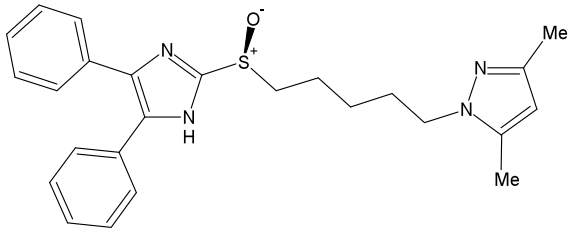
been successful and is used in the synthesis of the ACAT inhibitor RP 73163[3] **2** and potassium channel opener RP 52891[65,66] **50** by Rhône-Poulenc Rorer giving high ee values.



Scheme 1.23. *The Kagan method.*

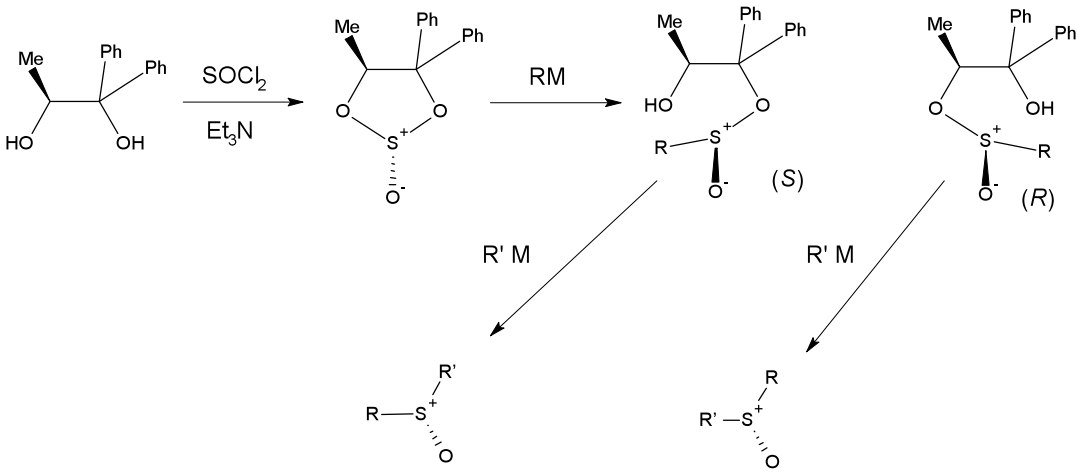


RP 52891 **50**



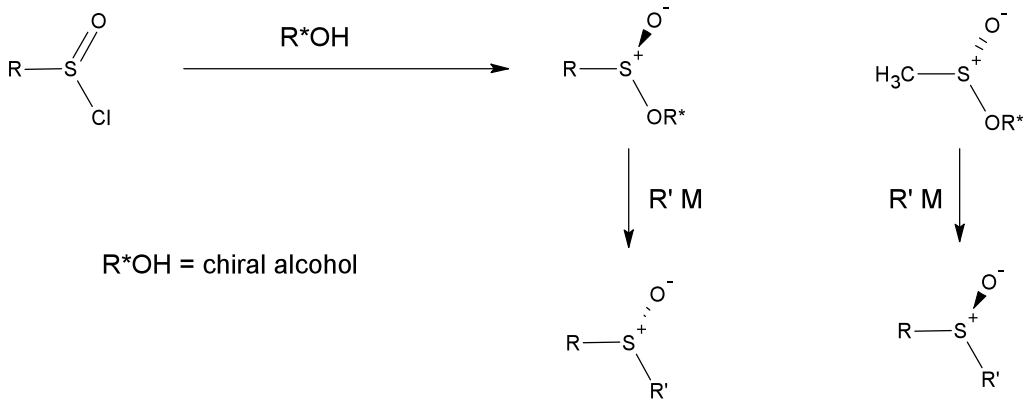
RP 73163 **2**

(ii) A second method developed by Kagan, involves formation of cyclic sulfites from a chiral diol and $SOCl_2$. This is followed by organometallic addition (usually a Grignard reaction) to give sulfinate diastereoisomers that are used in turn to obtain enantiomerically pure sulfoxides. Advantages of this method include the possible use of different organometallic reagents and the formation o dialkyl sulfoxides. Both enantiomers can often be produced since the diastereoisomers are readily separable in many cases (**Scheme 1.24.**).



Scheme 1.24. *Synthesis of sulfoxide enantiomers from cyclic sulfites*.

(iii) The 'Andersen method' was the first route available for the synthesis of enantiopure sulfoxides and also involves the formation and separation of a mixture of diastereoisomers. These were formed by reacting a sulfinyl chloride with a chiral alcohol (usually menthol), followed by an organometallic addition to the separated diastereoisomers, to give each enantiomer (**Scheme 1.25**.).



Scheme 1.25. *The Andersen method*.

A number of biological methods for the synthesis of chiral sulfoxides are available. Enzymes that will catalyse the asymmetric addition of an oxygen atom to thioethers include monooxygenases, dioxygenases, chloroperoxidases, and bromoperoxidases and the topic has been reviewed in a recent paper by Holland[67]. These may be found in whole cell organisms or as purified enzymes requiring co-factor addition and dioxygen or hydrogen peroxide. Enzymes are also available that

will kinetically resolve racemic mixtures of sulfoxides either by reduction to sulfide (deoxygenation) or oxidation to sulfone (oxygenation), with one enantiomer being removed faster than another.

1.7. Methods for determination of enantiomeric excess (ee).

1.7.1. Sulfoxides.

1.7.1.1 Chiral stationary phase HPLC.

Over the past 20 years a range of chiral stationary phases (CSPs) have been developed for the resolution of sulfoxide enantiomers. Among the most useful stationary phases used for the resolution of sulfoxides were those based on ester and carbamate derivatives of long chain carbohydrates (amylose or cellulose, see **Fig 1.8.**), hydrogen bonded on to a silica support. The instability of these columns, *e.g.* Chiralcel OB, has however been a major problem. An alternative superior type of column has the CSP covalently bonded to a silica support (**Fig 1.9.**).

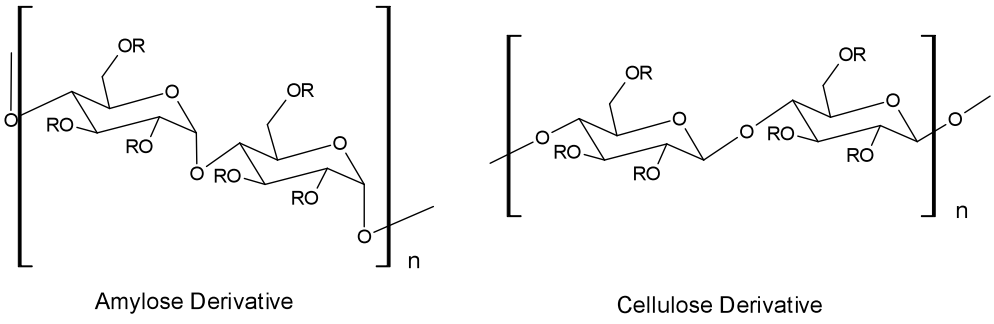


Fig 1.8. *Carbohydrate based stationary phases.*

In this category the (*S*,*S*)-Whelk-O1 column, with spherical Kromasil silica, is a chiral π-electron acceptor / π-electron donor phase, covalently bonded to the silica support. The major advantage of this covalently bonded support being that many solvent systems can be used with the column and the chiral phase will not normally be removed by the solvent.

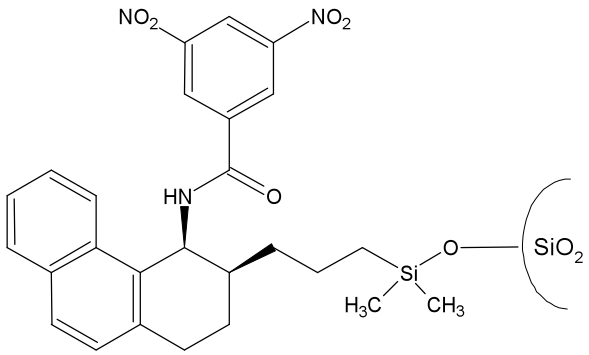
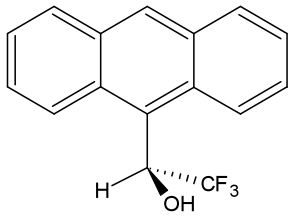


***Fig 1.9**. (S,S)-Whelk-O1 chiral stationary phase*.

1.7.1.2. NMR spectroscopy involving addition of chiral reagents.

Enantiomerically pure compounds which can complex with chiral sulfoxides may show distinguishable ^{1}H-NMR "diastereoisomeric"signals for each enantiomer. The most widely used reagent in this category is "*Pirkle solvent*", (+)-(*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol **51**. When one equivalent of this reagent is added to an NMR sample of racemic sulfoxide in $CDCl_3$ solution, two hydrogen bonded transient diastereoisomeric complexes can be formed (**Fig 1.10.**), which have different physical properties, including NMR chemical shift positions.



(+)-(S)-1-(9-anthryl)-2,2,2-trifluoroethanol **51**

In order to achieve baseline resolution of peaks in the NMR spectrum, the alkyl R_1 and R_2 groups should preferentially exist as a singlet.

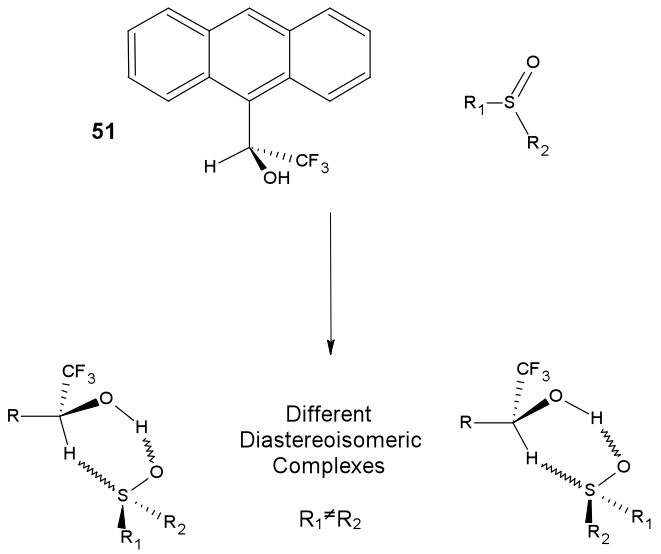


Fig 1.10. *Pirkle solvent/sulfoxide complexes.*

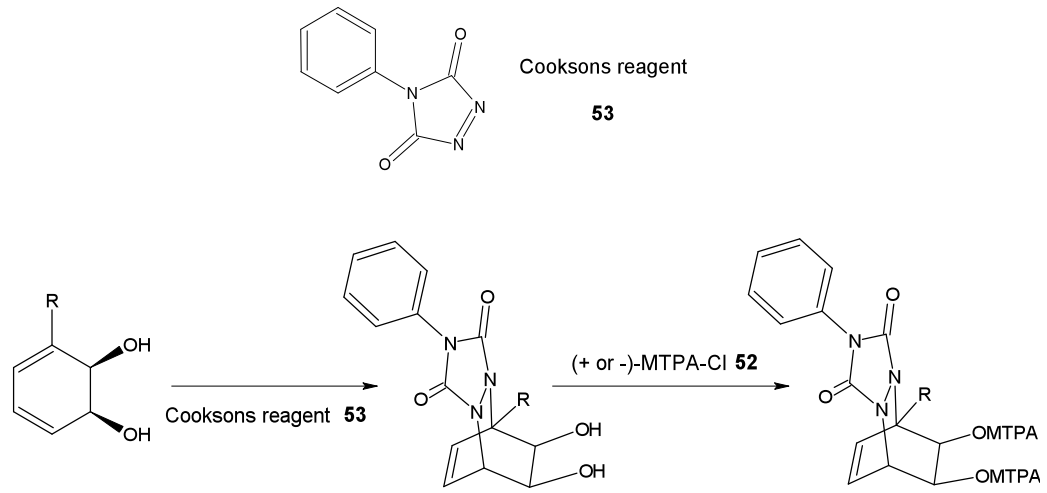
1.7.2. *cis*-Dihydrodiols.

1.7.2.1. MTPA Derivative formation.

The most widely used method for determination of enantiomeric purity of alcohols involves reacting one equivalent of either (+)- or (-)-methoxytrifluoromethyl phenyl acetyl (MTPA) chloride (+)- or (-)-**52** to the chiral alcohol (**Scheme 1.26.**) Formation of the MTPA (Mosher) ester followed by analysis using NMR spectroscopy and HPLC has been used in the determination of ee values. Three different NMR singlet signals associated with the MTPA group ($^{19}F_{CF_3}$, $^{1}H_{OMe}$, $^{1}H_{HA}$) are widely used as a measure of the ee values. Unfortunately due to the instability of *cis*-dihydrodiols and derivatised diols which readily aromatize, the MTPA method cannot be employed directly.

Scheme 1.26. *MTPA derivatisation.*

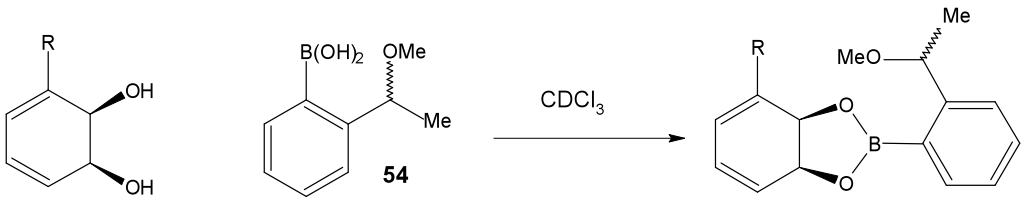
Stable MTPA esters can however be formed after selective hydrogenation of one C=C bond, or formation of cycloadducts. One method that has been used widely is the addition of Cooksons reagent (4-phenyl-4,5-dihydro-3*H*-1,2,4-triazole-3,5-dione) **53** to the *cis*-dihydrodiols (**Scheme 1.27.**). The addition of this reactive dienophile readily forms a cycloadduct exclusively *cis* to the diol groups, preventing premature decomposition and allowing derivatisation with the MTPA-Cl **50**.



Scheme 1.27. *Enantiomeric excess determination with Cooksons reagent* ***53***.

1.7.2.2. Chiral boronate formation.

cis-Dihydrodiols can also be derivatised using chiral boronic acids, such as 2-(1-methoxyethyl)benzene boronic acid (MEBBA) **54**, Unlike the MTPA esters, the MEBBA esters can be formed in a single step and are stable in $CDCl_3$, thus allowing direct NMR analysis. The sample can simply be dissolved in $CDCl_3$ in an NMR tube with addition of one equivalent of (*R*)- or (*S*)- chiral boronic acid to form a boronate ester (see **Scheme 1.28.**).



Scheme 1.28. *Boronate ester formation*.

1.8. Methods for determination of absolute configuration.

Throughout this thesis the absolute configurations of many compounds have been rigorously determined by X-ray diffraction methods. This technique is generally used if there is no more convenient way to determine the absolute configuration of a chiral compound. Excluding X-ray crystallography, the two most commonly used alternative methods, for the determination of absolute configurations are stereochemical correlation and circular dichroism (CD) spectroscopy. Empirical methods have also been used for the determination of absolute configuration of *cis*-dihydrodiols. These include cycloadduct-diMTPA formation or chiral boronate derivatisation followed by NMR analysis.

1.8.1. Stereochemical correlation methods.

In cases where the absolute configuration of known chiral compounds are being determined, a simple specific optical rotation ($[\alpha]_D$) measurement or chiral HPLC analysis may be sufficient. This involves correlating the correct configurations with sign and magnitude of rotation or enantiomer elution sequence on a CSP HPLC trace. This may also be extended to compounds of unknown absolute configuration. If a trend can be established for enantiomers of compounds from a topological series, giving similar signs of rotation ($[\alpha]_D$) or peak elution order *via* CSP HPLC, it may be possible to predict the absolute configuration of an enantiomer of an unknown compound of similar topology. It may also be possible to determine the stereochemistries of unknown compounds by chemically changing a functional group on a chiral molecule, without racemising or destroying the chiral centre, to produce a fully characterised compound of known absolute configuration; or by synthesising that molecule from one with known stereochemistry, using chemical steps which either do not affect the chiral centres involved, or alter them in a predictable fashion.

1.8.2. Circular dichroism spectroscopy.

CD spectroscopy can be used to determine the absolute configurations of members of a series of non-racemic chiral compounds. This technique is generally used if the absolute stereochemistry of one member of the series has already been established.

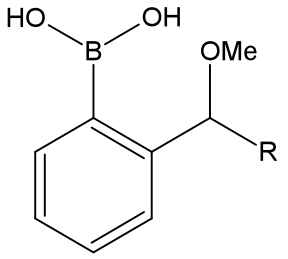
Linear polarised light consists of left [L] and right [R] circularly polarised components. When this light is not absorbed, *e.g.* at 589nm, but passed through a solution of a chiral enantioenriched compound, the plane of polarisation may be rotated to the left or right, depending on the configuration of the chiral compound, thus giving rise to positive and negative α- values. When an enantioenriched chiral compound, containing a chromophore, absorbs light, the absorption of the left and right components of polarised light are different. The difference in absorption (ε_L - ε_R), represented by $\Delta\varepsilon$ (absorption coefficient) changes over a range of wavelengths, giving positive and negative values. This is measured by θ, which can be used to determine $\Delta\varepsilon$ by the equation: $\theta \div 33 = \Delta\varepsilon \times c \times l$ (c = concentration, l = path length). A CD spectrum contains a plot of $\Delta\varepsilon$ *v* λ (wavelength) and will give both positive and negative curves. For similar types of compounds having the same configuration, the curves take a similar shape. Compounds of opposite configuration will have the same shape of CD curve but having opposite signs.

A strong positive or negative CD absorption is called a *'Cotton effect'*, and it has been observed that chiral centers, in conjunction with a certain chromophores, can produce predictable Cotton effects. In relation to sulfoxides, Andersen and Mislow [63,68-71] have observed that alkylaryl sulfoxides gave a strong Cotton effect at around 230 - 255nm (positive for *R* and negative for *S* configuration) and with dialkyl sulfoxides, Ohta *et al*[72] have observed a strong Cotton effect in the 215 - 225nm region which was positive for *S* and negative for *R* configurations. In the case of *cis*-dihydrodiols, a strong positive Cotton effect is usually located around 265 - 275nm, due to the interaction between the diene chromaphore and the C-1 and C-2 chiral centres; this is commonly associated with *cis*-(1*S*,2*R*)-dihydrodiols.

1.8.3. Chiral boronate Derivative formation.

The formation of chiral boronate derivatives can also be used in the determination of absolute configuration of *cis*-dihydrodiols, as well as in ee measurements (see **1.7.2.2.**). Initial work, carried out by Burgess and Porte[73] and later by Resnick *et al*[74] involved the derivatisation of a range of diols with chiral boronic acids. An empirical rule has been developed that enables the determination of absolute configuration of a *cis*-dihydrodiol boronate ester, from a comparison of the relative δ values of the ^{1}H-NMR signals of the two boronate diastereoisomers.

A range of alternative chiral boronic acids has been synthesised by Harrison and McConville in these laboratories.[75,76] These new chiral boronic acids were designed to suit diols of varying structures. These include 2-(1-Methoxyethyl)Benzene Boronic Acid (MEBBA) **54**, 2-(1-Methoxy-2-Methylpropyl)Benzene Boronic Acid (MMBBA) **55**, 2-(1-Methoxy-2,2-Dimethylpropyl)Benzene Boronic Acid (MDBBA) **56**, 2-(1-Methoxy-1-Phenylmethyl)Benzene Boronic Acid (MPBBA) **57** and 2-(1-Methoxy-2,2,2-Trifluoroethyl)Benzene Boronic Acid (MTBBA) **58**.



R = Me (MBBA) **54**
R = Pr^i (MMBBA) **55**
R = Bu^t (MDBBA) **56**
R = Ph (MPBBA) **57**
R = CF_3 (MTBBA) **58**

Chapter 2 Enzyme-catalysed enantioselective deoxygenation of chiral sulfoxides.

As the research programme in this thesis was mainly directed towards synthesis of chiral sulfoxides, the two different approaches of asymmetric synthesis and kinetic resolution, involving different sets of enzymes, have been used. The most appropriate method would then be used for the synthesis of specific types of sulfoxide.

2.1. Available biological approaches to chiral sulfoxides.

2.1.1. Asymmetric synthesis.

Whole cell fungal and bacterial systems have been used to enantioselectively oxidise sulfides to chiral sulfoxides.[77-92] Recent work carried out by this research group[30,90,93] into the TDO- and NDO- catalysed metabolism of a range of alkyl aryl and diaryl sulfides has shown that dioxygenases have the ability to introduce chirality not only by *cis*-dihydroxylation, but also by enantioselective sulfoxidation. A few typical examples illustrate the range of products from dioxygenase-catalysed oxidation (**Table 2.1.**). Methyl phenyl sulfide **23** when metabolised by *P. putida* UV4 (TDO) gave the sulfoxide **(*R*)-24** as a single product (> 98% ee and 90% yield), from oxidation at a position α-to the aromatic ring. (2-Fluorophenyl)methyl sulfide **59** when metabolised by *P. putida* UV4 gave sulfoxide **(*R*)-60** in (> 98% ee and 43% yield) and also a *cis*-dihydrodiol **61** (> 98 % ee and 7% yield), due to attack at the α-position and at the arene ring. 2,3-Dihydrobenzo[*b*]thiophene **62** was a substrate with *P. putida* UV4 and gave sulfoxide **(*R*)-63** (26% ee, 5% yield) and hydroxysulfide **(*S*)-64** (90% ee, 8% yield) both from attack at positions α- to the ring. Phenyl(2-thienyl) sulfide **65**, containing both a heterocyclic and an acyclic sulfur atom, gave the diaryl sulfoxide **66** (66% ee, 15% yield) and the unstable thiophene sulfoxide **67** as a minor metabolite from *S. yanoikuyae* B8/36 (BPDO). These bioproducts demonstrate the different scope of reactions catalysed by dioxygenases but also show the limitations of the enzymes. Attack occurred mainly at positions α- to the aromatic ring, but also at the ring itself. In all cases sulfoxidation was observed but with variable ee values (often > 98%). Enantiocomplementarity was observed using different dioxygenase-containing strains. One disadvantage of using dioxygenases for sulfoxidation was that

this type of enzyme could not normally oxidise sulfur atoms unless they were directly attached to an aromatic ring, or formed part of an aromatic heterocyclic ring.

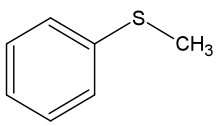
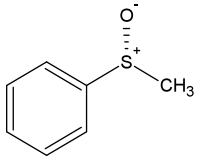
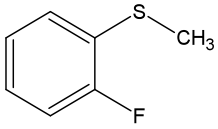
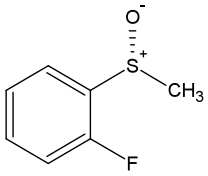
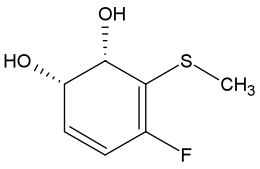
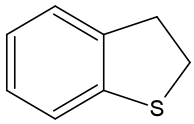
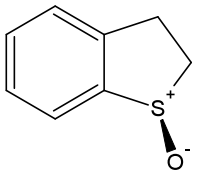
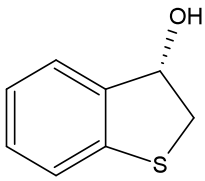
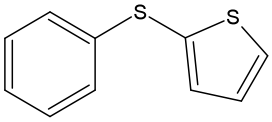
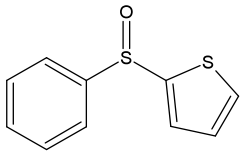
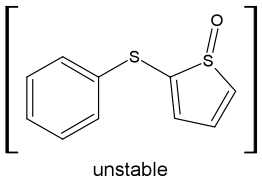
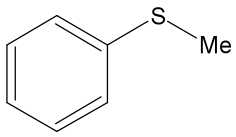
SUBSTRATE	STRAIN (ENZYME)	BIOPRODUCT 1	BIOPRODUCT 2
 **23**	*P. putida* UV4 (TDO)	 **(*R*)-24** > 98% ee	n/a
 **59**	*P. putida* UV4 (TDO)	 **(*R*)-60** > 98% ee	 **(+)-61** > 98% ee
 **62**	*P. putida* UV4 (TDO)	 **(*S*)-63** 26% ee	 **(*S*)-64** 90% ee
 **65**	*S. yanoikuyae* B8/36 (BPDO)	 **66**	 unstable **67**

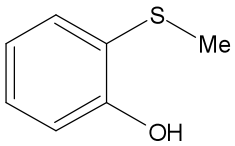
Table 2.1. *Dioxygenases-catalysed oxidation of aryl sulfides* ***23, 59, 62*** *and* ***65****.*

Dioxygenases have also failed to oxidise aromatic compounds containing hydroxyl (phenols) or amino (anilines) groups. For example, while methyl phenyl sulfide **23** was oxidised to sulfoxide **24** in a dioxygenase-mediated process, (2-hydroxyphenyl)methyl sulfide **68** was not. These deficiencies prompted the current

investigation into other methods for creating enantiopure chiral sulfoxides. A main focus was thus directed towards the kinetic resolution of racemic mixtures of sulfoxides.



Methylphenyl sulfide **23**



(2-hydroxyphenyl)methyl sulfide **68**

2.1.2. Kinetic resolution of sulfoxides.

Kinetic resolution of sulfoxides using enzymes may involve different approaches including hydrolysis of esters, reduction of ketones and oxidation to sulfones. One literature example of enzymatic kinetic resolution of a series of chiral organosulfur compounds (Colonna *et al*[94]) involves the resolution of cyclic sulfites by enantioselective oxygenation to the sulfates catalysed by cyclohexanone monooxygenase (CMO). In this paper,[94] a series of 5- and 6-membered cyclic sulfite rings were enantioselectively oxygenated, giving ee values up to > 99% in two cases and > 90% in four cases.

Sulfoxide deoxygenation provides a further method and has been used in the current study. Enzymatic deoxygenation of sulfoxides has been known to occur for more than thirty years. A paper published in 1981 by Auret *et al*[95] demonstrated that the enzymatic deoxygenation of both 1,3-dithiane-1-oxide and 1,3-dithiane-1,3-dioxide to yield 1,3-dithiane could be achieved using reductase enzymes present in the fungus *Mortierella isabellina* NRRL 1757. This fungal species, normally associated with asymmetric sulfoxidation of sulfides, was thus also capable of asymmetric deoxygenation of sulfoxides. When racemic 1,3-dithiane-oxide was used as substrate, the recovered material was found to have a low ee value. The kinetic resolution of a racemic sulfoxide through biotransformation at the sulfoxide centre, can be achieved either by a process of enantioselective oxygenation to yield sulfone, or deoxygenation of the sulfoxide to yield sulfide. In the present study DMSO reductases were used to catalyse the enantioselective deoxygenation reaction. Two previous examples in the literature of kinetic resolution of chiral sulfoxides, using reductase enzymes were

provided by Abo *et a.l*[96,97] The earlier reference[96] described the preparation of enantioeriched sufoxides from strains of *Rhodobacter sphaeroides* . This strain was obtained from the waste water of a 'tofu' factory and contained DMSO-reductases capable of enantioselectively reducing racemic sulfoxides. The later reference[97] describes the electrochemical enzymatic deoxygenation of chiral sulfoxides using purified DMSO reductase from *Rhodobacter sphaeroides*. This method allowed greater control over the reduction of various functionalised sulfoxides, which gave poor yields with the whole-cell organism, by avoiding undesirable side reactions.

The kinetic resolution technique, utilising DMSO reductase enzymes involves the selective removal of a single sulfoxide enantiomer from a racemic mixture leaving the residual sulfoxide enriched in one enantiomer and giving the corresponding sulfide. The reductase enzymes can achieve this enrichment when one of the enantiomers is more reactive to deoxygenation than the other (**Fig 2.1.**). It is also assumed that no oxygenation (sulfoxidation) of the product sulfide can occur.

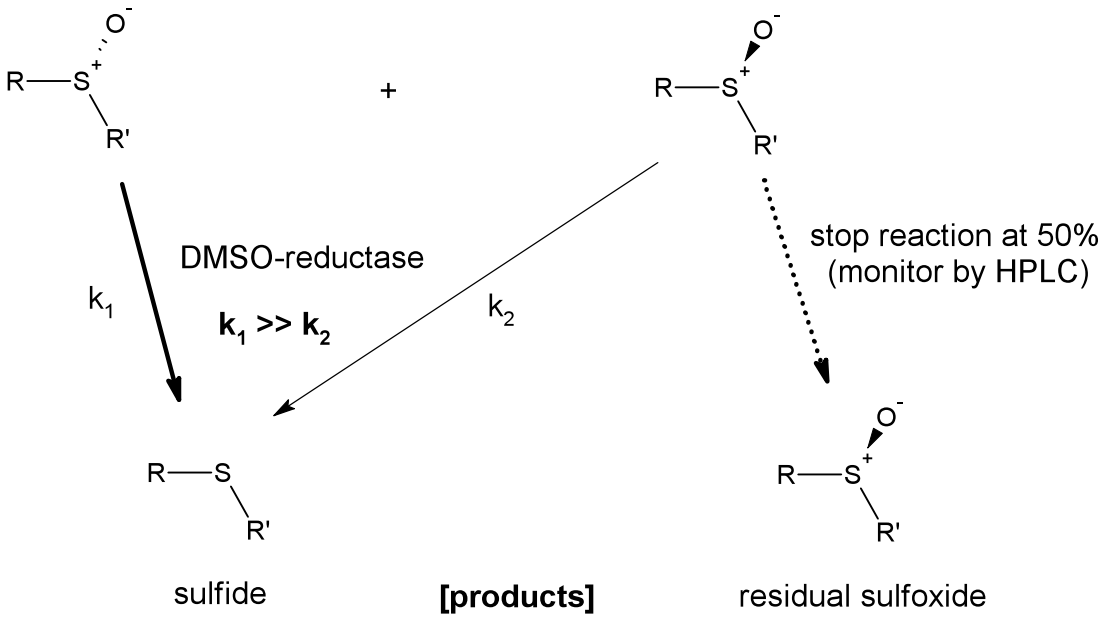


Fig 2.1. *Kinetic resolution of racemic sulfoxides with DMSO-reductase*.

Ideally the reaction should be monitored by HPLC and stopped at approximately 50% conversion. Depending on the relative reactivities of each enantiomer to deoxygenation, a longer biotransformation period may be required for some racemates. This however is undesirable as the yields are already at best 50%. A

typical reverse phase HPLC trace of the crude culture medium from the biotransformation, shows the appearance of the sulfide peak and the disappearance of the sulfoxide peak during the resolution of racemic methyl-*p*-tolyl sulfoxide (MPTSO) **29** using *Proteus vulgaris* (**Fig 2.2.**). This biotransformation was carried out at the University of Warwick as part of a collaborative study. Prior to this biotransformation study, suitable analytical CSP HPLC systems were developed which allowed the estimation of the % ee of the residual sulfoxides (**Table 2.2. & Appendices**).

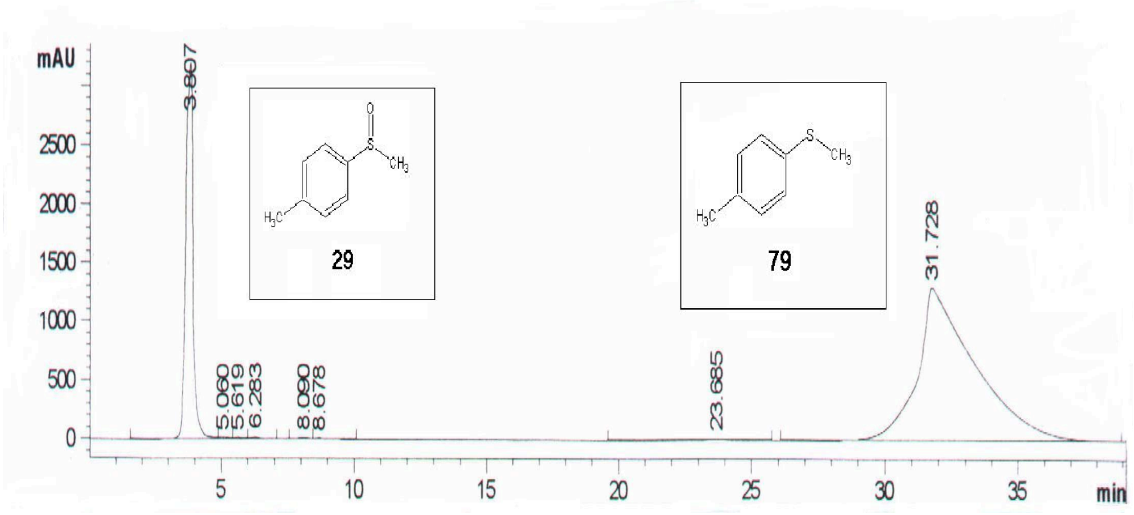


Fig 2.2. *C-18 reverse phase HPLC trace of the biotransformation media from MPTSO resolution with Proteus vulgaris.*

In principle, synthesis of chiral sulfoxides by selective deoxygenation of sulfones would be synthetically useful as this process would be an asymmetric synthesis rather that a kinetic resolution with a theoretical yield of 100% for a single sulfoxide enantiomer. Unfortunately this deoxygenation reaction did not occur when tested on methyl-*p*-tolyl sulfone **69** (**Scheme 2.1.**); virtually all of the sulfone was recovered.

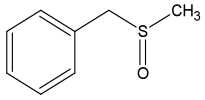
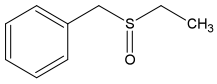
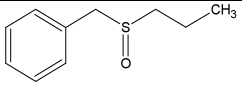
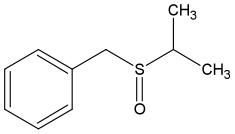
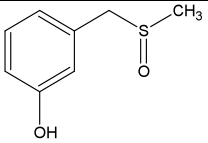
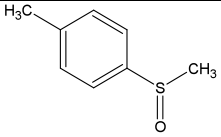
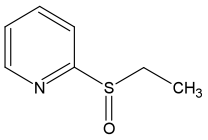
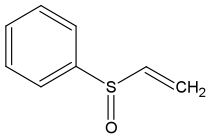
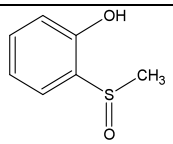
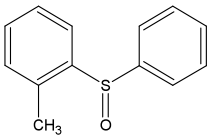
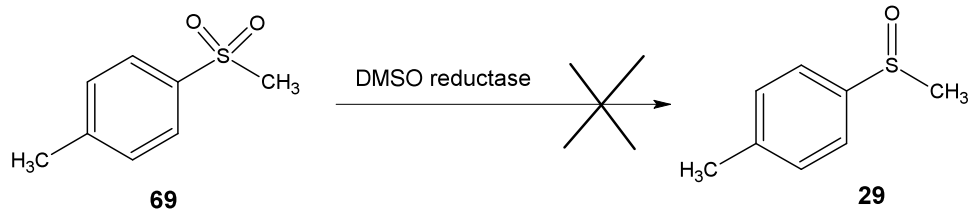
Compound	Peak Elution / min		Column / Conditions	α-value
	S-sulfoxide	*R*-sulfoxide		
 **70**	36	46	Chiracel OB 10% IPA/Hex 0.5cm^3/min	1.32
 **71**	30	35	As above	1.17
 **72**	28	30	As above	1.07
 **73**	27	29	As above	1.06
 **74**	32	36	As above	1.13
 **29**	20	60	As above	3.1
 **75**	19	37	As above	1.95
 **76**	34	50	As above	1.47
 **77**	19	30	As above	1.58
 **78**	15	13	Chiralcel OJ 10% IPA/Hex 0.7cm^3/min	1.15

Table 2.2. *CSP HPLC separation and retention times of sulfoxide enantiomers.*



69 **29**

Scheme 2.1. *Inability of DMSO reductase to catalyse the deoxygenation of sulfone* **69**.

On hindsight this result was not suprising since it is established that chemical deoxygenation reactions are much slower on sulfones compared with sulfoxides. Furthermore, a comprehensive search of the literature failed to provide any evidence of this reaction having occurred under enzyme catalysis.

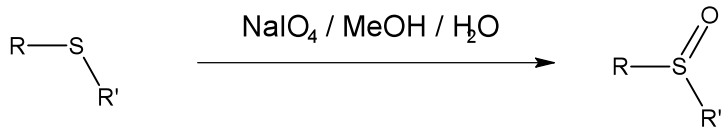
2.2. Selection and synthesis of substrates, for DMSO reductase enzymes.

As the whole-cell reductase-catalysed biotransformations were carried out in the Department of Biological Sciences at the University of Warwick (Drs C. Walker and H. Luckarift), there was a limit to what control could be exercised over the monitoring of the biotransformations from these laboratories. HPLC, using a reverse phase C-18 column, was found to be the most convenient method for monitoring the progression of the reactions from direct analysis of the culture medium. This method was not completely satisfactory as both the substrates and products have differing solubilities in the aqueous reaction media. The less water-soluble sulfide products were more likely to precipitate from solution and could thus appear to be present in lower concentrations in the culture medium. Volatile sulfide products (*e.g.* thiophenes) also had the potential to be removed from solution by evaporation, while some sulfide bioproducts were unstable (*e.g.* 1,2-disulfides). For these reasons, a completely accurate measure of the reaction progress was difficult to obtain. In view of these experimental difficulties, it was decided to screen a range of substrates using several reductase enzymes, and to determine the potential of this method for the synthesis of chiral sulfoxides. Of particular interest in this project, was the possible application of these enzymes in the synthesis of functionalised chiral sulfoxides, which have proved difficult to obtain using other enzyme systems, *e.g.* dioxygenases.

Representative substrates were chosen ranging from dialkyl sulfoxides, alkyl aryl sulfoxides, diaryl sulfoxides, thiophene sulfoxides, hydroxy sulfoxides and a sulfinate.

2.2.1. General procedures for the synthesis of sulfoxides.

Several sulfides and sulfoxides were commerically available or had been previously synthesised. These included methyl-*p*-tolyl sulfide **79**, ethyl-2-pyridyl sulfide **80**, benzylmethyl sulfide **81**, *o*-hydroxythioanisole **68**, dibenzo[*b,d*]thiophene **82** and phenylvinyl sulfoxide **76**. The sulfides were then converted to the sulfoxides using one of the several available oxidation procedures. These methods included sodium periodate, $NaIO_4$, which was used for oxidising the alkylbenzyl sulfides, phenyl-*o*-tolyl sulfide **83**, ethyl-2-pyridyl sulfide **80** and methyl-*p*-tolyl sulfide **79**. This was the favoured oxidation method for these sulfides, which were methanol soluble. Furthermore, the reagent was neutral, the workup was simple, the yields were good (> 75%) and no sulfone was produced, simplifying their purification (**Scheme 2.2.**).



R = Bz, R' = Me	**70**
R = Bz, R' = Et	**71**
R = Bz, R' = Pr	**72**
R = Bz, R' = Pr^i	**73**
R = *p*-Tolyl, R' = Me	**29**
R = *o*-Tolyl, R' = Ph	**78**
R = 2-Pyridyl, R' = Et	**75**

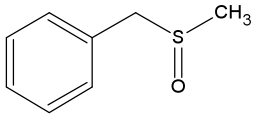
***Scheme 2.2**. Sodium periodate oxidation to sulfoxides **29, 70-73, 75** and **78**.*

Meta-Chloroperoxybenzoic acid (MCPBA) and dimethyldioxirane (DMD) provided convenient oxidation procedures for sulfides requiring stronger oxidants. DMD was a particularly useful oxidant since the reaction occurred rapidly at ambient temperature giving almost quantitative yields. The byproduct was acetone which was also the solvent allowing for a simple workup procedure. This was important in cases where the sulfoxides produced were unstable under acidic conditions or elevated

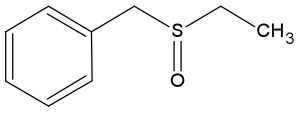
temperatures. For reactions where MCPBA overoxidation to the sulfone was possible, mixtures of MCPBA with borontrifluoride etherate (BF_3-Et_2O) or MCPBA with hexa fluoroisopropanol (HFIP) mixtures were used. The yields obtained were close to quantitative and no sulfone was produced. These methods were used to oxidise thiophenes to the corresponding thiophene oxides. Sulfoxidation of the thiophene is generally slow since formation of the sulfoxide will result in a considerable loss of resonance energy. The non-aromatic thiophene sulfoxide can then be readily oxidized to the corresponding thiophene sulfone without any further significant decrease in resonance energy. The use of particular oxidants will be discussed in the context of the synthesis of individual sulfoxides.

2.2.2. Synthetic routes to dialkyl sulfoxides.

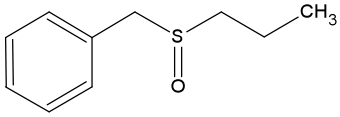
The dialkyl sulfoxides selected were benzylmethyl sulfoxide (**70**), benzylethyl sulfoxide (**71**), benzylpropyl sulfoxide (**72**), benzylisopropyl sulfoxide **73,** (2-hydroxybenzyl)methyl sulfoxide **84** and (3-hydroxybenzyl)methyl sulfoxide **75**.



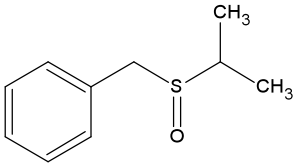
70



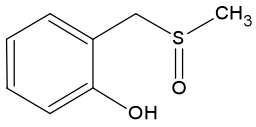
71



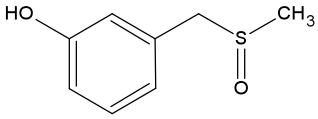
72



73



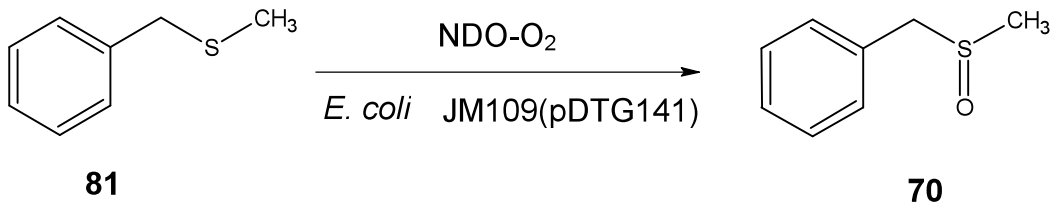
84



74

The inclusion of an aromatic ring in each case resulted in decreased problems with the volatility of the sulfoxides or sulfides. The presence of a benzene chromophore also made the extraction, analysis and purification of the enantioenriched sulfoxide easier. The use of dioxygenases for the synthesis of enantiopure alkylbenzyl sulfoxides had previously proved to be very inefficient, giving low yields and ee values. In the case of the biotransformation of benzylmethyl

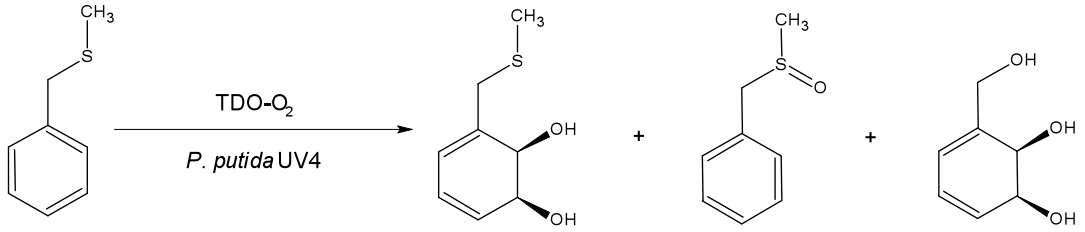
sulfide **81** using the NDO-containing strain, *E. coli* JM109 (pDTG141) Holland *et al*[83] isolated racemic sulfoxide in 20% yield and with a very low ee (< 5%, **Scheme 2.3.**).



81 **70**

Scheme 2.3. *Biotransformation of benzylmethyl sulfide* ***81*** *with E. coli JM109(pDTG141).*

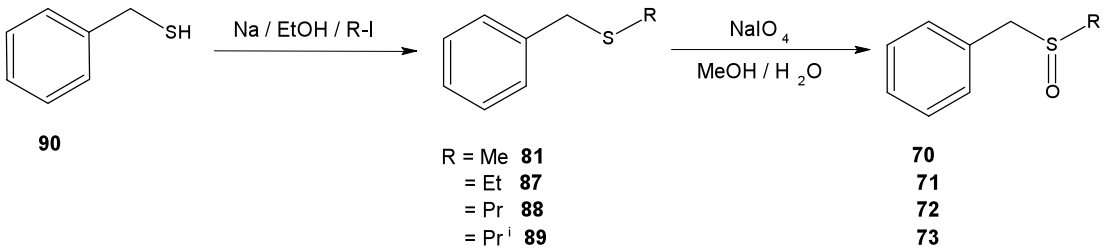
From an earlier biotransformation of benzylmethyl sulfide **81** with *P. putida* UV4,[86] several bioproducts were reported including racemic sulfoxide **70** (**Scheme 2.4.**). The other bioproducts were *cis*-(1*S*,2*S*)-3-(methylthio)methyl cyclohexa-3,5-diene-1,2-diol **85**, a dihydroxylation product and *cis*-(1*S*,2*S*)-3-(hydroxy)methyl cyclohexa-3,5-diene-1,2-diol **86**, a trihydroxylation product.



81 90% relative yield **85** 8% yield **70** 2% yield **86**

Scheme 2.4. *Biotransformation of benzylmethyl sulfide* ***81*** *with P. putida UV4.*

The synthesis of sulfoxide substrates **70-73** was completed using the literature methods. Thus, the sulfides **87**, **88**, and **89** were synthesised by deprotonation of benzyl thiol **90** using sodium ethoxide, followed by alkylation using the appropriate alkyl halide. The sulfides were then purified by column chromatography or distilled and sulfoxidised using $NaIO_4$ (**Scheme 2.4.**).



90

R = Me **81**
= Et **87**
= Pr **88**
= Pr^{i} **89**

70
71
72
73

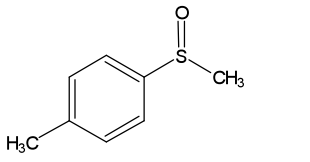
Scheme 2.4. *Synthesis of alkylbenzyl sulfoxides **70-73**.*

The CSP HPLC peaks corresponding to individual enantiomers present in racemic sulfoxides **70-73** were separated using a Chiralcel OB column (10% IPA/Hex, flow 0.5cm^3/min). The separation was indicated by the α values of 1.32 for benzylmethyl (**70**), 1.17 for benzylethyl (**71**), 1.07 for benzylpropyl (**72**) and 1.06 for benzylisopropyl sulfoxides **73**. The efficiency of the enantiomer separation was thus found to decrease with increasing size of the alkyl chain (**Appendicies 2.1.-2.4.**).

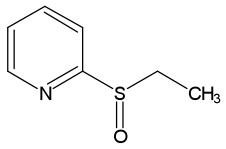
The synthesis of racemic samples of the phenolic dialkyl sulfoxides **84** and **74** was attempted using several different approaches. Unfortunately these methods were unsuccessful. However, small samples of enantioenriched sulfoxides **74** and **84** were obtained by aromatisation of the corresponding *cis*-diol sulfoxide metabolites (See later). Partial enantiomer separation by CSP HPLC analysis has been observed for sulfoxide **74** using a Chiralcel OB column (10% IPA/Hex, flow 0.5cm^3/min, α 1.13).

2.2.3. Alkylaryl sulfoxides.

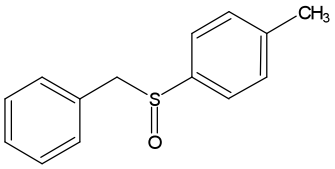
The alkylaryl sulfoxides selected for kinetic resolution studies were methyl-*p*-tolyl sulfoxide **29**, ethyl-2-pyridyl sulfoxide **75**, methyl-*o*-hydroxyphenyl sulfoxide **77** and benzyl-*p*-tolyl sulfoxide **91**. These sulfoxides were chosen as they contain differing functionalities, including a pyridyl ring in sulfoxide **75**, and a phenolic group in sulfoxide **77**. Compound **91**, a slightly larger bicyclic sulfoxide, was selected as a substrate since it will later be used in a chiral synthesis (**Chapter 3**). Sulfoxide **29** was selected as a model since it had previously been used as a substrate enzyme-catalysed deoxygenation reaction. It would also allow a comparison to be made with all the DMSO reductase-containing strains or pure enzymes.



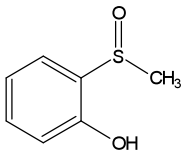
29



75

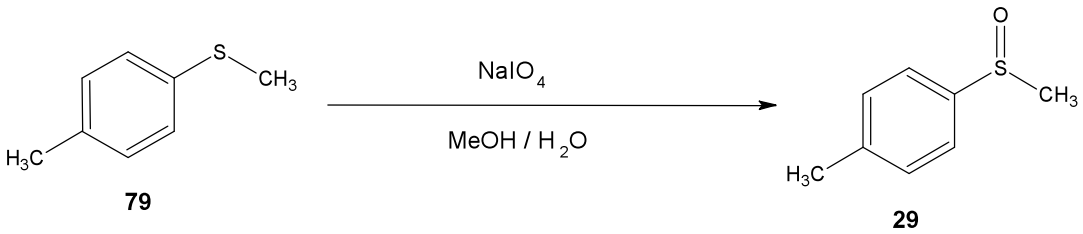


91



77

Methyl-*p*-tolyl sulfoxide **29** was synthesised by $NaIO_4$ oxidation of the commerically available sulfide **79** and gave the racemic sulfoxide in a 90% yield (**Scheme 2.5.**).

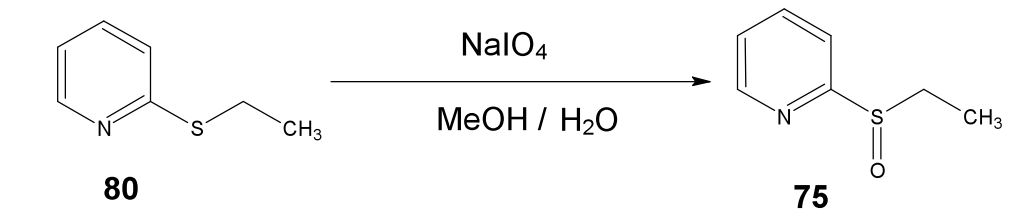


79 **29**

Scheme 2.5. *$NaIO_4$ oxidation of a sulfide **79**.*

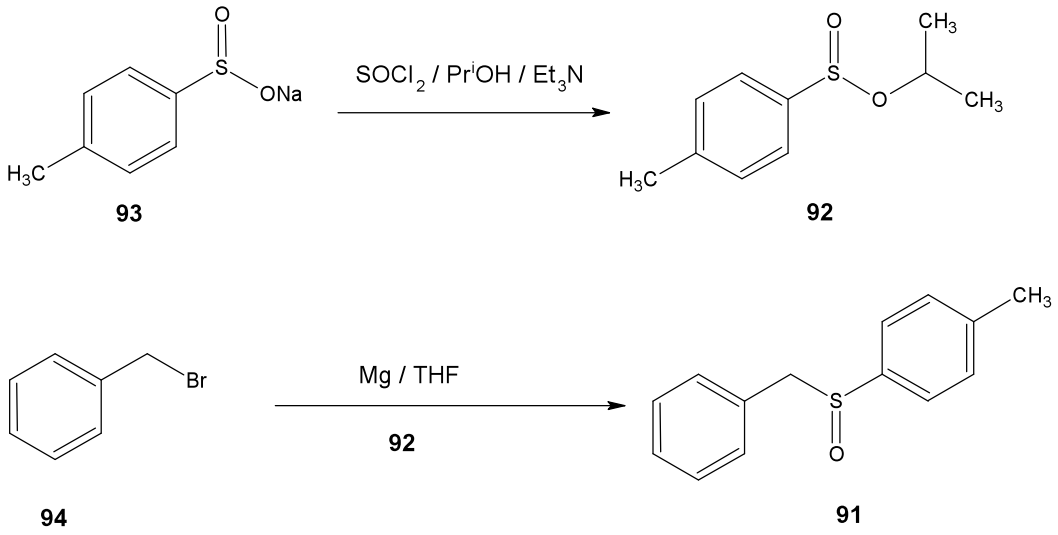
A CSP HPLC separation of racemic sulfoxide enantiomers **29** was achieved using a Chiralcel OB column (10% IPA/Hex, flow 0.5cm^3/min, α 3.11) (**Appendix 2.6.**).

Ethyl-2-pyridyl sulfoxide **75** was also synthesised by $NaIO_4$ oxidation of the sulfide **80** giving the sulfoxide in an 81% yield. (**Scheme 2.6.**) Separation of the enantiomers of sulfoxide **80** was again observed using a Chiralcel OB column (10% IPA/Hex, flow 0.5cm^3/min, α 2.33) (**Appendix 2.7.**).



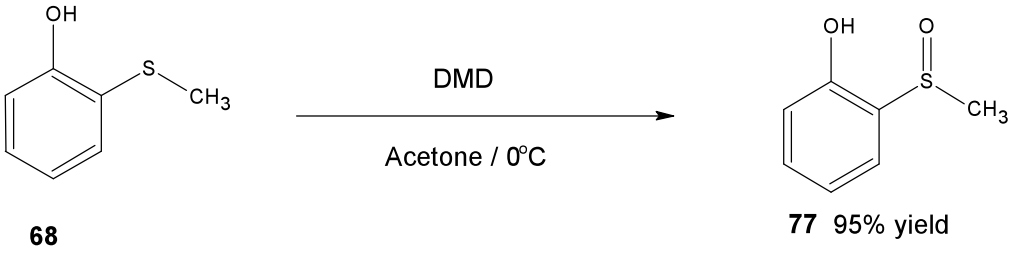
Scheme 2.6. *$NaIO_4$ oxidation of a sulfide* ***80***.

Benzyl-*p*-tolyl sulfoxide **91** was synthesised from isopropyl-*p*-tolyl sulfinate **92**. This was obtained in a 98% yield from the sodium salt of *p*-tolyl sulfinic acid **93** using thionyl chloride and triethylamine in IPA. A Grignard reaction was then carried out on sulfinate **92** using benzyl magnesium bromide (generated from benzyl bromide **94**) giving the sulfoxide **91** in a 97% yield (**Scheme 2.7.**).



Scheme 2.7. *Synthesis of benzyl-p-tolyl sulfoxide* ***85***.

The sodium periodate sulfoxidation method was not suitable for *o*-hydroxythioanisole **68** as this oxidant has been found to oxidise phenols. In this case DMD was the best oxidant. This oxidant was formed from the reaction of acetone with sodium bicarbonate and oxone (potassium peroxymonosulfate), was used to oxidise the commerically available sulfide **68** to yield sulfoxide **77** in a 95% yield (**Scheme 2.8.**).



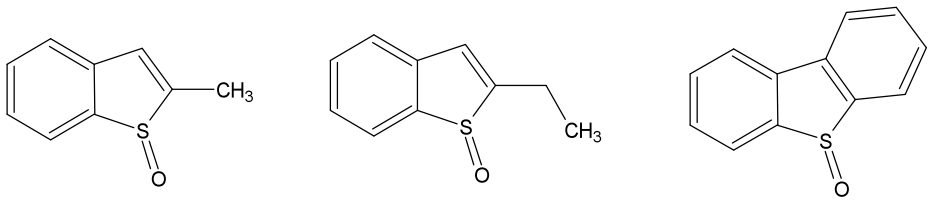
68 **77** 95% yield

Scheme 2.8. *DMD oxidation of a sulfide* ***68***.

CSP HPLC analysis of the racemic sulfoxide **77** was carried out using a Chiralcel OB column (10% IPA/Hex, flow 0.5cm^3/min, α 1.58, **Appendix 2.8.**).

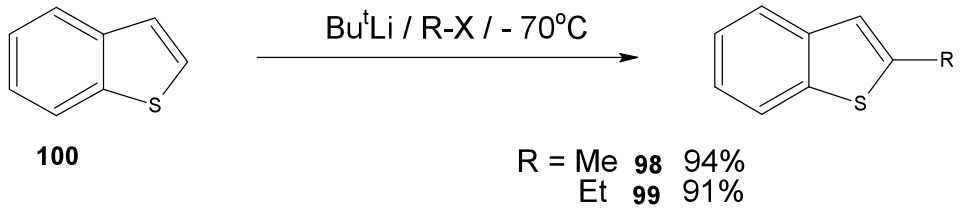
2.2.4. Thiophene oxides.

Due to the instability of most monocyclic thiophene oxides and unsubstituted benzo[*b*]thiophene oxides (which undergo cycloaddition reactions), more stable substituted benzothiophene and dibenzothiophene oxides were used. Three configurationally stable thiophene oxides, 2-methylbenzo[*b*]thiophene oxide **95**, 2-ethylbenzo[*b*]thiophene oxide **96** and dibenzo[*b,d*]thiophene oxide **97** were used. Although the tricyclic sulfoxide **97** was not chiral, it was considered to be a good model thiophene since it was also chemically stable but was more readily deoxgenated. It would also test if larger substrates of this type could be accepted into the active site and reduced by these enzymes. Sulfoxides **95** and **96** were the smallest stable chiral thiophene oxides that could be used for these studies.



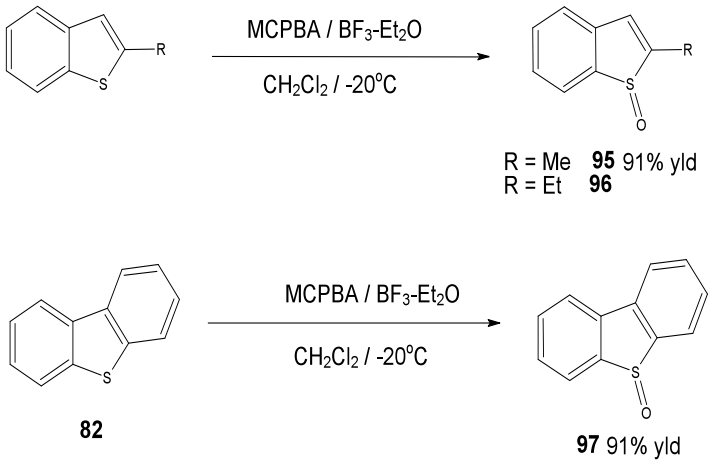
95 **96** **97**

The 2-substututed benzo[*b*]thiophenes **98** and **99** were formed by reacting benzo[*b*]thiophene **100** with Bu^{t}Li forming a carbanion exclusively at the 2- position. This nucleophile was then reacted with the corresponding alkyl halide to give the products **98** and **99** in 94% and 91% yields respectively (**Scheme 2.9.**).



***Scheme 2.9**. Synthesis of alkylbenzo[b]thiophenes **98** and **99**.*

Neither $NaIO_4$ nor DMD were suitable oxidants for the sulfoxidation of the thiophenes **98**, **99** or dibenzo[*b,d*]thiophene **82**. The periodate reagent was not strong enough to oxidise thiophene rings while DMD was too powerful and sulfone formation was also observed. The best reagent for thiophene sulfoxidation this was a mixture of MCPBA/BF_3-Et_2O in CH_2Cl_2 at - 20°C which had been used earlier[98] giving > 90% yields (**Scheme 2.10.**). The formation of a complex between the sulfur atom of the thiophene and BF_3 appeared to allow sulfoxidation but also prevented further oxidation to the corresponding sulfone.

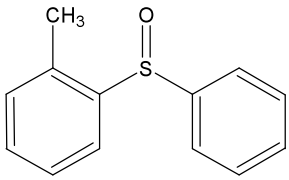


***Scheme 2.10.** Sulfoxidation of thiophenes **82, 98** and **99**.*

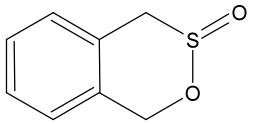
Thiophene oxide **95** was found to have sufficient configurational stability to allow separation into enantiomers by CSP HPLC using an (*S,S*)-Kromasil Whelk-01 column (50% MeOH/Water, flow 1.0cm^3/min, α 1.14, **Appendix 2.9.**).

2.2.5. Diaryl sulfoxide 78 and sulfinate 101.

The racemic diaryl sulfoxide **78** was used to determine the enantioselectivity of the DMSO reductase where there was little difference between the size and shape of the phenyl and *o*-tolyl substutuents. The racemic sulfinate chosen as a potential substrate was benzo[*d*]-1,2-oxithian-1-oxide **101**.



78



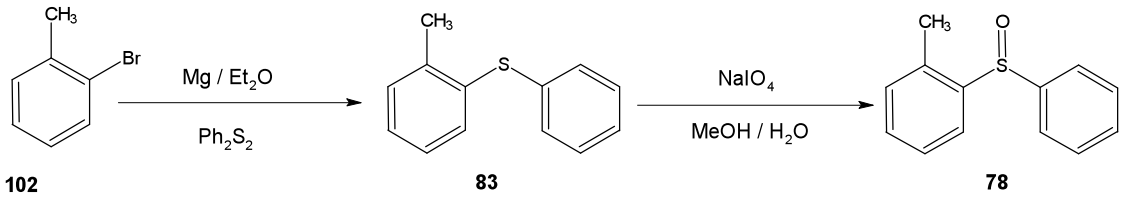
101

As discussed in Chapter 1, chiral sulfinates can be used in the synthesis of single enantiomer sulfoxides. This approach was applied during the Andersen and Kagan methods (**Chapter 1**), where the sulfinate reacted with an alkylating organometallic reagent (**Scheme 2.11.**). Sulfinate (**101**) was chosen as it was stable under the biotransformation conditions and had an additional chromophore with the inclusion of the aromatic ring.

101

Scheme 2.11. *Synthesis of hydroxy sulfoxides from sulfinate **101**.*

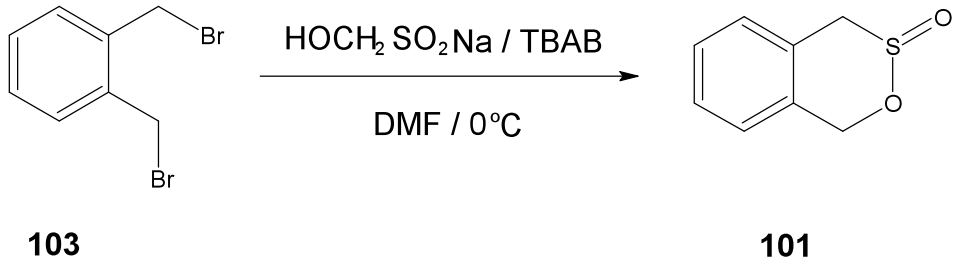
Sulfide **83** was synthesised by reacting diphenyldisulfide with a Grignard reagent formed from *o*-bromotoluene **102**. Sulfide **83** was then oxidised using $NaIO_4$ to yield phenyl *o*-tolyl sulfoxide **78** (**Scheme 2.12.**).



***Scheme 2.12**. Synthesis of phenyl-o-tolyl sulfoxide **78**.*

A separation of the enantiomers of sulfoxide **78** was achieved by CSP HPLC analysis using a Chiralcel OJ column (10% IPA/Hex, flow 0.7cm^3/min, α value 1.10, **Appendix 2.10.**).

The sulfinate **101** was synthesised from 1,2-dibromomethylbenzene **103** with 2-hydroxymethyl sodium sulfinate and tetrabutylammonium bromide (TBAB) in DMF (**Scheme 2.13.**).



***Scheme 2.13.** Synthesis of sulfinate **101**.*

CSP HPLC analysis using a Kromasil (*S*,*S*)-Whelk-01 semi-prep column (Bu^{t}OMe, flow 2.5cm^3/min) provided a separation of enantiomers of the sulfinate **101**, with an α value of 1.67 (**Appendix 2.11.**).

2.3. Biotransformations using DMSO reductase enzymes.

DMSO reductase-containing bacteria that were used in the biotransformation of racemic sulfoxides included *Rhodobacter capsulatus*, *Proteus vulgaris*, *E. coli*

HB101 and three strains of *Citrobacter braakwi* (DMSO-7, DMSO-10 and DMSO-11).

When the biotransformations were carried out the first steps involved monitoring the reaction by HPLC, until about 50% conversion of the sulfoxide had occurred. At this point the reaction was terminated by centrifugation and addition of EtOAc. The reductase biotransformation work was carried out at the University of Warwick. Both the initial synthetic work and the development of CSP HPLC systems were carried in Belfast as part of the current programme. The final steps in the process were also carried out at Belfast, and involved the extraction, purification and stereochemical analysis. The bioproducts were isolated from the aqueous media, either by removal of the water and extraction of the residue with organic solvent (EtOAc), or by saturation of the aqueous culture medium with salt and solvent extraction. The method involving removal of water under vacuum at 35-40 °C gave better yields. However if the bioproducts were unstable or volatile the second procedure was preferred. ^{1}H-NMR and TLC analysis of the crude extract were good indicators of the progress of the reaction. The presence of sulfide bioproduct was detected in the ^{1}H-NMR spectrum and by TLC analysis as the less polar metabolite. The more polar sulfoxide substrate was pre-purified before CSP HPLC analysis, as the crude extract often contained impurities that could diminish the column efficiency. PLC (silica-gel) was used to purify the sulfoxide prior to CSP HPLC separation of enantiomers which was then used to confirm if resolution had occurred, to determine the ee value and to identify the residual enantiomer. Absolute configurations of sulfoxides were determined using CSP HPLC, by comparison with known literature CSP HPLC separations[90,97,99,100] or by comparison with enantiopure samples of sulfoxide of known configuration (**Appendices**).

From a total of fifteen racemic sulfoxides (**29**, **70-78**, **84**, **91** and **95-97**) and a sulfinate **101** synthesised for this study, five (**71-73** and **76**) showed little evidence of kinetic resolution or production of sulfide, with one (**84**) showing complete conversion to sulfide, when a *Citrobacter* strain (DMSO-11) was used. Enantiomers of sulfinate **101**, which had never been produced in enantiopure form before, were resolved using a semi-prep. Kromasil (*S,S*)-Whelk-01 column. Unfortunately it has not yet been possible to determine the configurations of the enantiomers. The $[\alpha]_D$ values (+ 68, $CHCl_3$ for the early peak and – 70, $CHCl_3$ for the later peak) were,

within experimental error, of equal magnitude and of opposite sign. The CD spectra of these compounds were also recorded (**Fig 2.3.**) showing absorption peaks at 205nm and 226nm.

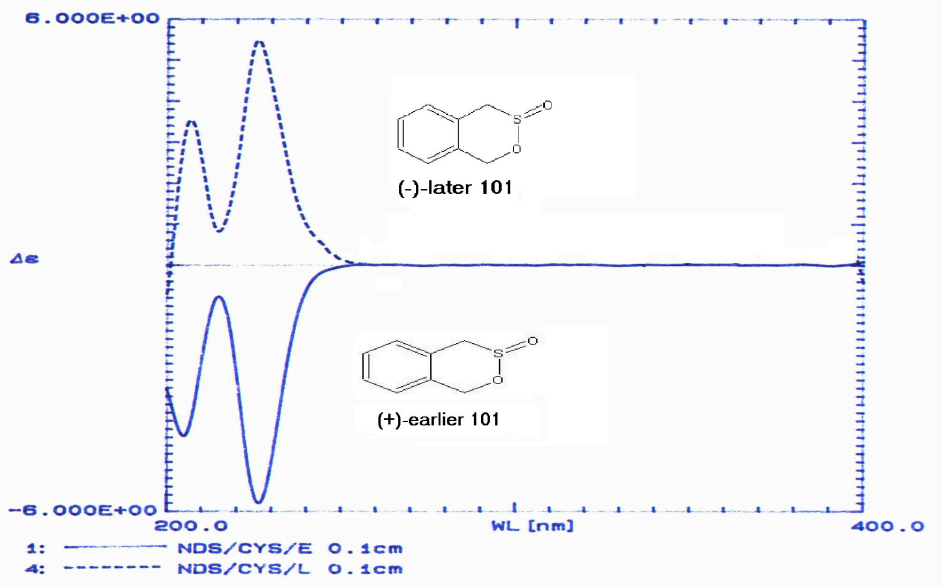


Fig 2.3. *CD spectra corresponding to (+)- and (-)-enantiomers of sulfinate* ***101****.*

To assess the enantioselectivity of the available DMSO reductase-containing strains, the alkylaryl and dialkyl sulfoxides methyl *p*-tolyl sulfoxide **29** and benzylmethyl sulfoxide **70** were chosen. The results obtained with the *R. capsulatus*, *P. vulgaris*, *E. coli*, *C. braakwi* (DMSO-7, DMSO-10 and DMSO-11) strains are shown in **Table 2.2.**. These results indicated that (i) using sulfoxides **29** and **70**, a degree of kinetic resolution has been achieved with all strains, (ii) the most efficient total kinetic resolution of sulfoxide **29** has been found with strains *P. vulgaris* and *C. braakwi* DMSO-10 giving the (*S*)-methyl-*p*-tolyl sulfoxide (*S*)-**29**, (iii) the *E. coli* and *C. braakwi* DMSO-11 strains also gave an excess of **(***S*) enantiomer of sulfoxide **29** with the remaining *R. capsulatus*, and *C. braakwi* DMSO-7 strains produce the (*R*)-sulfoxide **(*R*)-29**, and (iv) using sulfoxide **70**, all strains showed the same enantioselective preference giving (*S*)-benzylmethyl sulfoxide **(*S*)-70** with the best results (77% ee) from the *P. vulgaris* strain. The results in **Table 2.2.** provide only a preliminary survey of the potential of the different bacterial strains, but it is clear that

the degree of enantioselectivity associated with deoxygenation using different DMSO reductases can change with different substrates.

Sulfoxides **74**, **75**, **77, 78** and **84** were metabolised with the DMSO-11 strain of *C. braakwi* in an attempt to assess the applicability of this DMSO reductase in the kinetic resolution of functionalised sulfoxides, containing phenolic (**74**, **77**, and **84**), and pyridyl substituents (**75**).

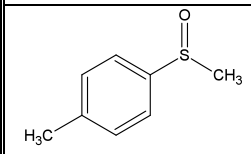
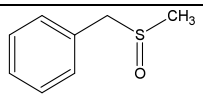
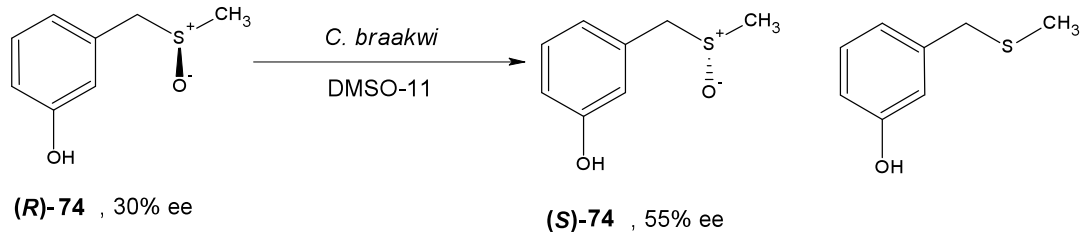
Sulfoxide	*R. capsulatus*	*P. vulgaris*	*E. coli*	*C. braakwi*		
				DMSO-7	DMSO-10	DMSO-11
 **29**	58% ee (*R*)	98% ee (*S*)	60% ee (*S*)	54%ee (*R*)	98%ee (*S*)	32% ee (*S*)
 **70**	–	77% ee (*S*)	68% ee (*S*)	44% ee (*S*)	37% ee (*S*)	26% ee (*S*)

Table 2.2. *Enantiomeric excess values and absolute configurations of residual sulfoxides **29** and **70** obtained from DMSO reductase-containing strains*.

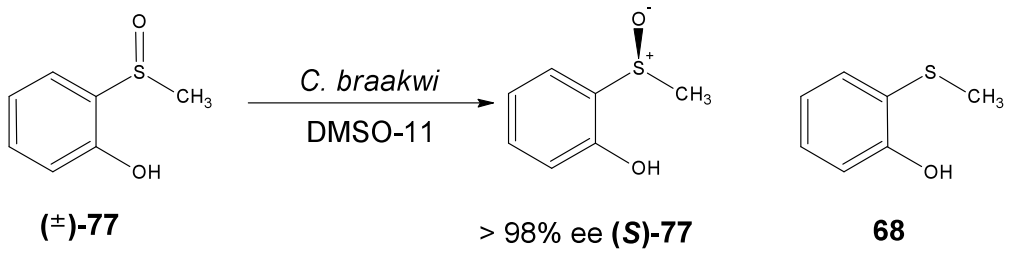
Phenolic sulfoxides **74**, **77** and **84** were metabolised, on a small scale, using DMSO-11. In the case of sulfoxide **84**, 100% conversion was observed, with recovery of the sulfide. This result indicates that sulfoxide **84** is an excellent substrate for DMSO reductase. Phenol sulfoxide **74** (30% ee, *R*) was added as substrate to bacterial culture of DMSO-11. The residual sulfoxide was mainly (*S*)-3-methylsulfinylmethyl phenol **(*S*)-74** with an ee of 55% indicating a stereopreference for the *R* enantiomer (**Scheme 2.14.**).



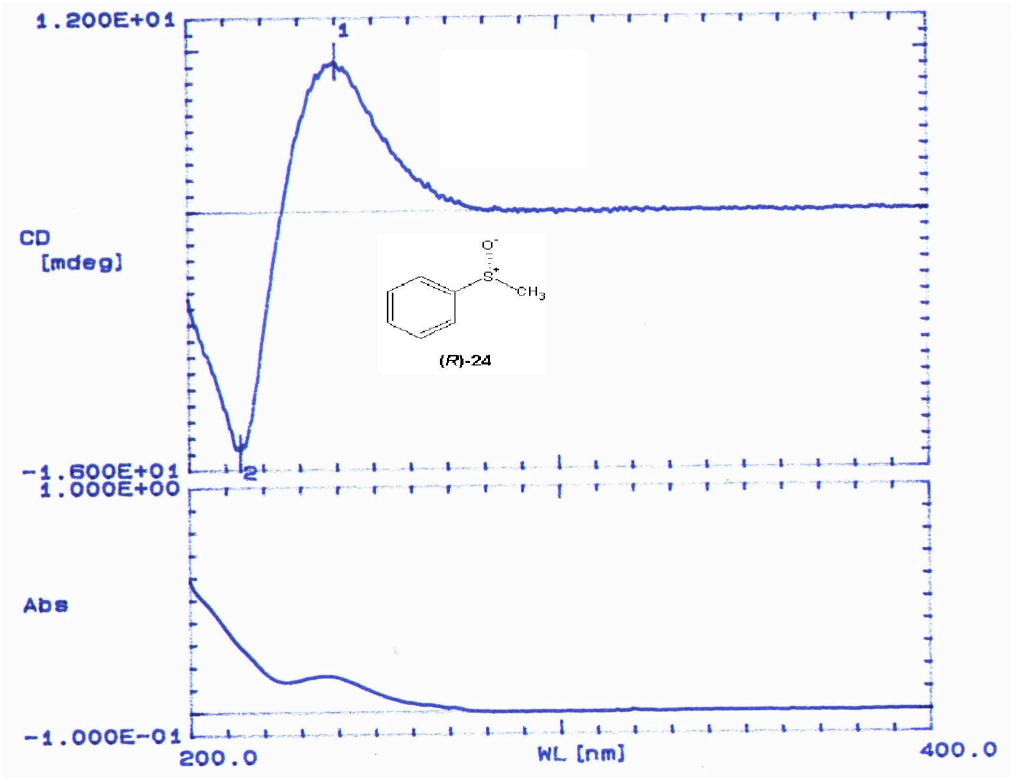
Scheme 2.14. *Kinetic resolution of sulfoxide **74**.*

Sulfoxide **78** was metabolised using *C. braakwi* DMSO-11, giving sulfide **83** and residual sulfoxide **(*S*)-78** with an ee value of 76%, showing that the new DMSO-11 strain has the ability to distinguish between substituents of only slightly differing sizes.

On a larger scale, racemic sulfoxides **75** (2g) and **77** (0.5g) were metabolised with *P. vulgaris* and DMSO-11 respectively. Sulfoxide **77** was biotransformed using DMSO-11 and the reaction was terminated at ~ 55% conversion. The sulfide bioproduct **68** was isolated and the residual enantiopure (> 98%ee) 2-methylsulfinyl phenol **77** was recovered in 27% yield (**Scheme 2.15.**). An optical rotation of was recorded ($[\alpha]_D$ – 189, $CHCl_3$) which confirmed the enantiopurity of the sulfoxide stereocentre as *S*, by comparison to the literature value[101]. The stereochemistry, which was previously unknown, at the sulfoxide centre was confirmed through CD correlation of the purified residue with a sample of enantioenriched (*R*)-methylphenyl sulfoxide **(*R*)-24** (**Fig 2.4.**), obtained from the *P. putida* UV4 metabolism of sulfide **23**. The CD spectra show strong, and opposite absorbtions at *ca:* 235-240nm and 215nm, showing the sulfoxide residue from DMSO-11 to be **(*S*)-77**.



Scheme 2.15. *Synthesis of enantiopure phenol sulfoxide **(S)-77**.*



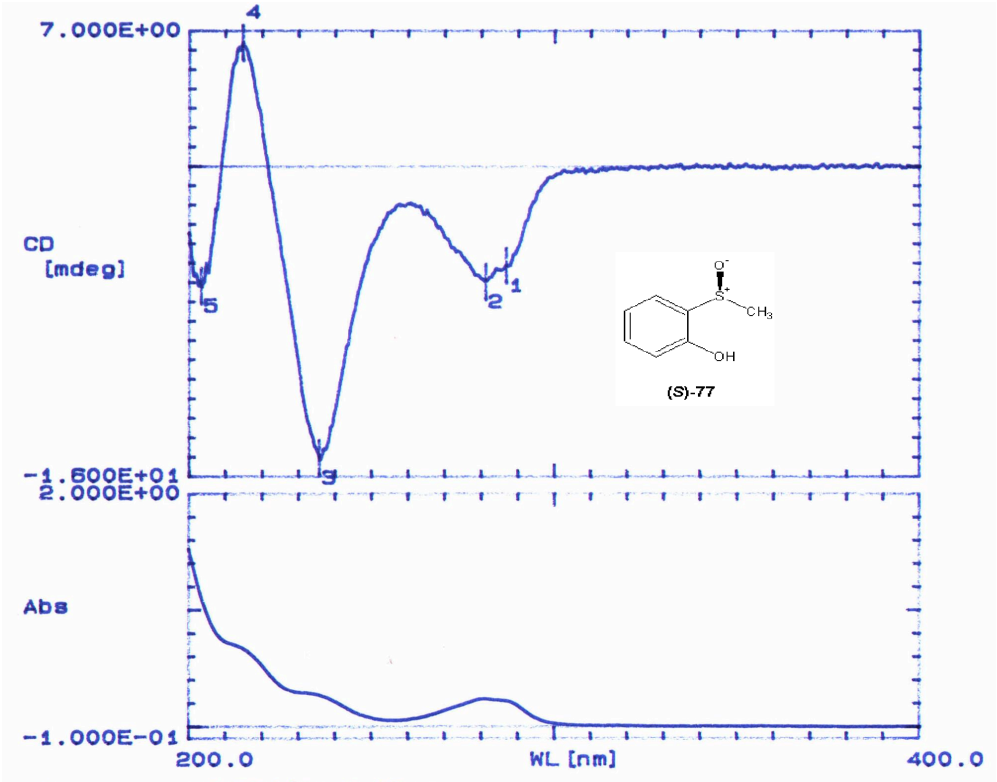
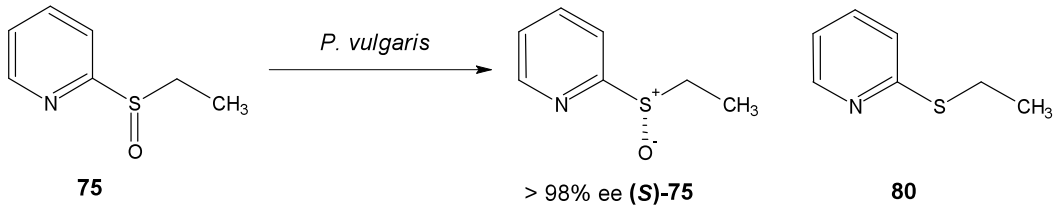


Fig 2.4. *Determination of the stereochemistry of (-)-(S)-2-methylsulfinyl phenol* ***(S)-77*** *by CD spectroscopy.*

Ethyl-2-pyridyl sulfoxide **75** was metabolised using *P. vulgaris*. The reaction was stopped at ~ 55% conversion. The sulfide bioproduct **80** was isolated after chromatography. Enantiopure (> 98%ee) (-)-(*S*)-ethyl-2-pyridyl sulfoxide **(*S*)-75** was recovered (45% yield) (**Scheme 2.16.**). The optical rotation of the residual sulfoxide was recorded ($[\alpha]_D$ -157, MeOH) and this confirmed the configuration of the sulfoxide stereocentre as (*S*).

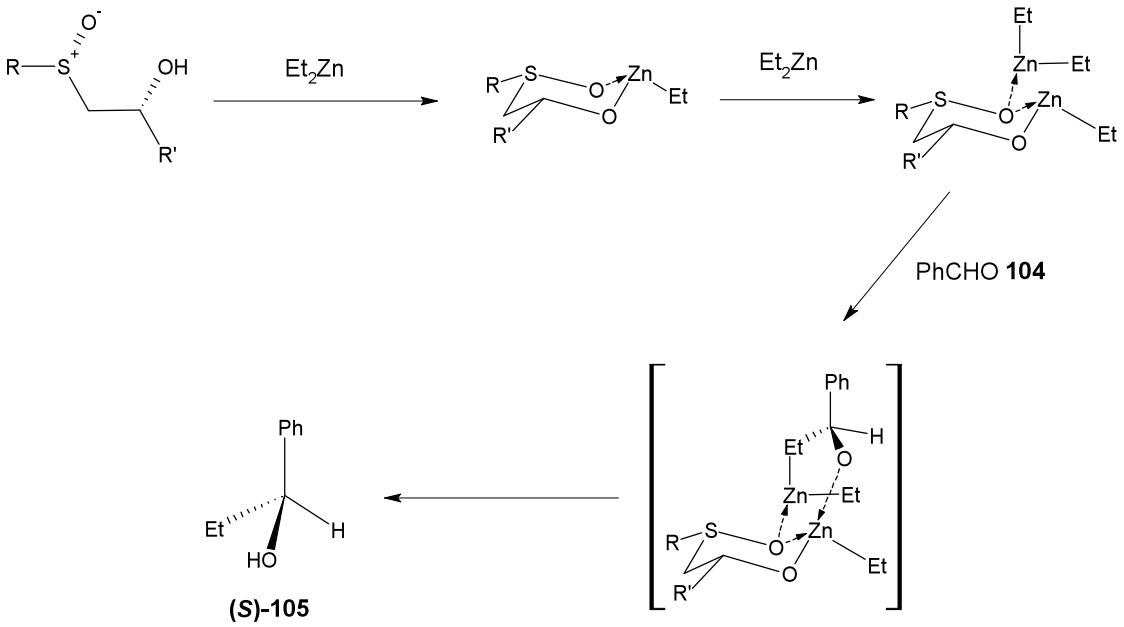


Scheme 2.16. *Synthesis of enantiopure pyridyl sulfoxide* ***(S)-75***.

The *P. vulgaris* and *C. braakwi* DMSO-11 strains were chosen for biotransformation of substrates **75** and **77**, as they had previously been shown to produce these enantiopure sulfoxides on a small scale. These particular substrates were selected due to their potential as chiral synthons. They also provide a good demonstration of the value of DMSO reductases in the synthesis of functionalised chiral sulfoxides in comparison with other enzyme systems.

2.4. Synthetic applications of sulfoxides obtained by DMSO reductase biotransformations.

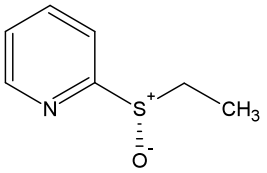
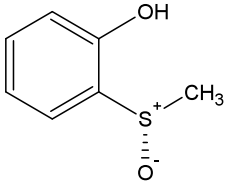
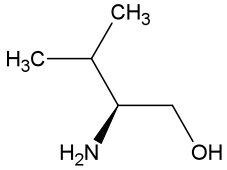
The enantioselective addition of diethylzinc (Et_2Zn) to carbonyl compounds has been catalysed by a range of chiral ligands including aminoalcohols,[102] diamines,[103] diols[104] and aminothiols.[105] As hydroxy sulfoxides have the ability to coordinate as bidentate ligands due to the availibility of lone pairs on each of the oxygen atoms, they have previously been reported to catalyse this reaction.[106] In this context, Et_2Zn was postulated to coordinate with the alcohol sulfoxide catalyst to form a complex, which was capable of catalysing enantiospecific addition of Et_2Zn to benzaldehyde **104** (**Scheme 2.17.**).

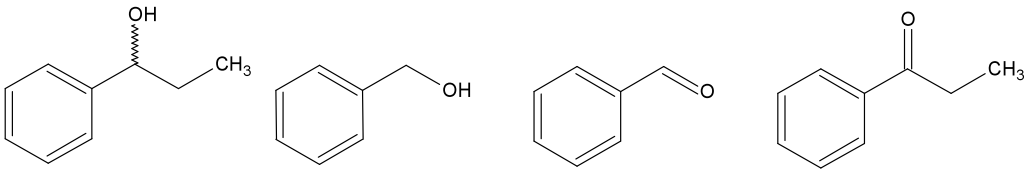


Scheme 2.17. *Catalytic addition of Et_2Zn to benzaldehyde **104**.*

The highest ee values (55%) reported for alcohol **105** was obtained by using a hydroxysulfoxide.[106] Other chiral ligands such as aminoalcohols have proved to be more successful as catalysts, although it seems that the best yields are always obtained using benzaldehyde **104** as substrate. Other aldehydes and ketones were alkylated with lower ee values. From this earlier study it is clear that there is potential for considerable improvement in this asymmetric alkylation reaction, especially in the synthesis of efficient catalysts containing enantiopure sulfoxide ligands.

To assess the potential of sulfoxides **75** and **77**, in this reaction, samples of the enantiopure sulfoxides **(*S*)-75** and **(*S*)-77** were studied. The experiments were carried out according to the procedures reported by Ogumi *et al*,[102] with a comparison of the sulfoxide catalysts **(*S*)-75**, **(*S*)-77** and the amino alcohol, (*S*)-valinol **106** (**Table 2.3.**), which had previously been used for this reaction giving (*R*)-phenylpropan-1-ol **105** with a 47% ee.

Ligand	Ee (%) of 105	Isolated Yield (%)			
		107	108	104	109
 **(*S*)-75**	0	47	5	26	–
 **(*S*)-77**	27.0	30	21	17	10
 **(*S*)-106**	8.7	79	8	0.5	–



107 **108** **104** **109**

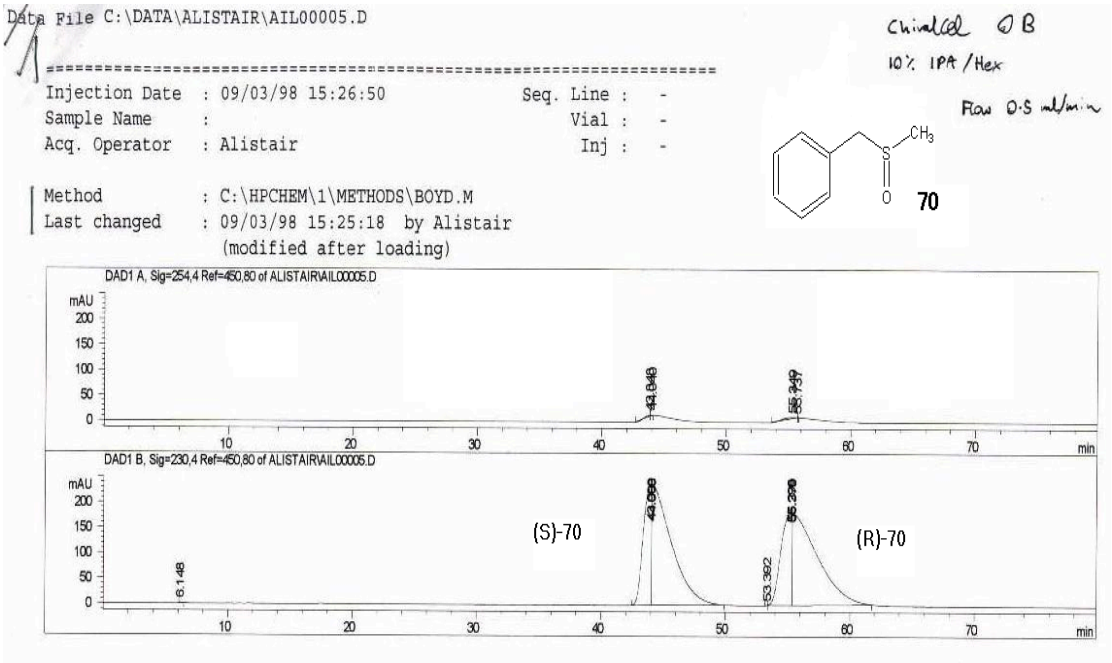
Table 2.3. *Enantioselective addition of Et_2Zn to benzaldehyde **104**.*

The results in **Table 2.3.** show that the ee values obtained are generally low, with sulfoxide **75** giving no enantiomeric excess. There is also a greater tendency towards side reactions with the yields of compounds **107**, **108** and **109** increasing compared to those for catalyst **(*S*)-106**. This however is not surprising as the conditions have not been optimised for chiral ligands of this type (*i.e.* containing alcohol and sulfoxide groups). In comparison to compound **(*S*)-106,** which was reported in the literature as giving a much higher ee value, the value obtained for sulfoxide **(*S*)-77** (27%) seems to be more promising.

2.5. Conclusion.

This study has demonstrated that using various strains of bacteria containing DMSO reductase, it is possible to kinetically resolve sulfoxides with enantiocomplementarity being evident, using different strains. New strains of *Citronella braakwi* (DMSO-7, DMSO-10 and DMSO-11) have been isolated and found to (resolve or partially resolve) functionalised sulfoxides such as phenolic sulfoxides, that are difficult to synthesise *via* the dioxygenase-catalysed sulfoxidation route. A hydroxy sulfoxide **(*S*)-77** obtained by kinetic resolution of the racemic sulfoxide using *Citronella braakwi* DMSO-11, has been demonstrated to have potential as a chiral ligand in the enantioselective alkylation of benzaldehyde **104**. using Et_2Zn.

Appendix 2.1.



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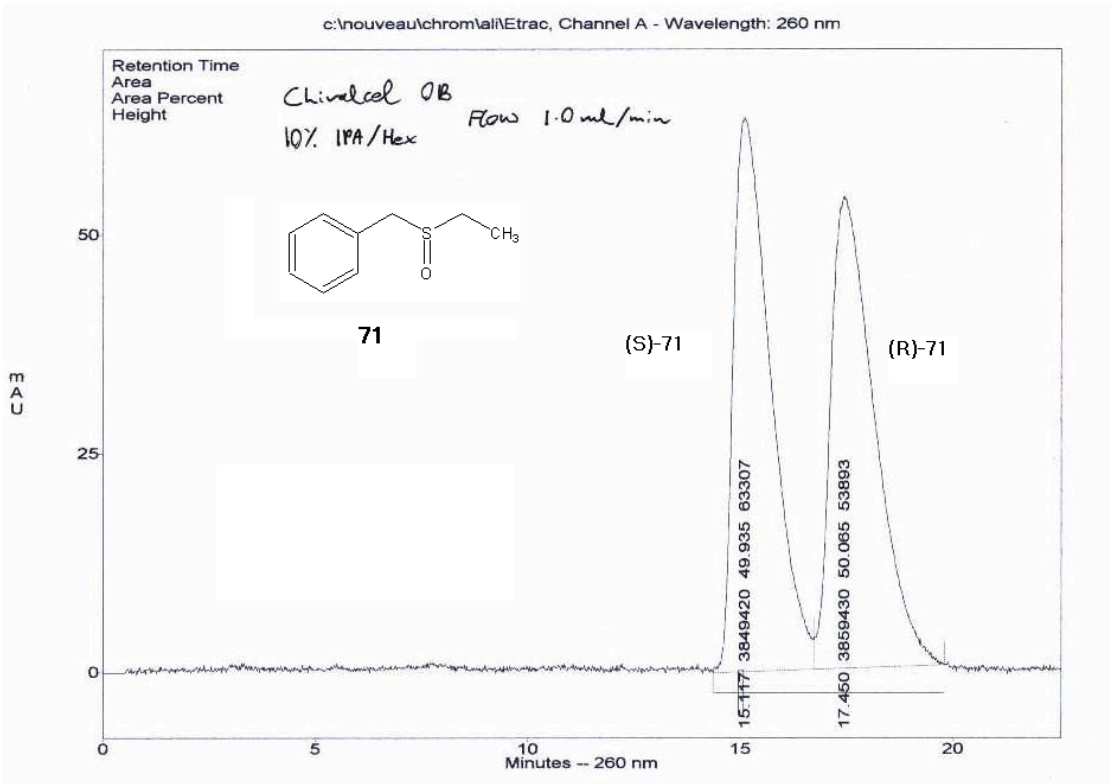

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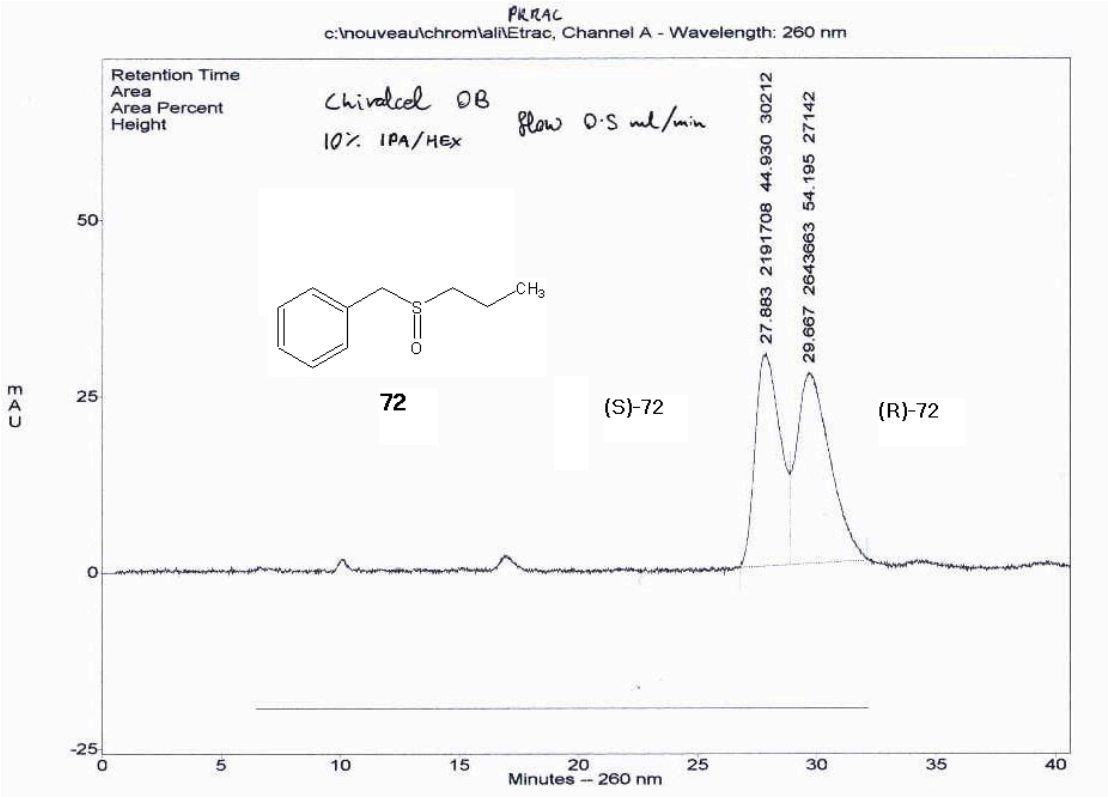

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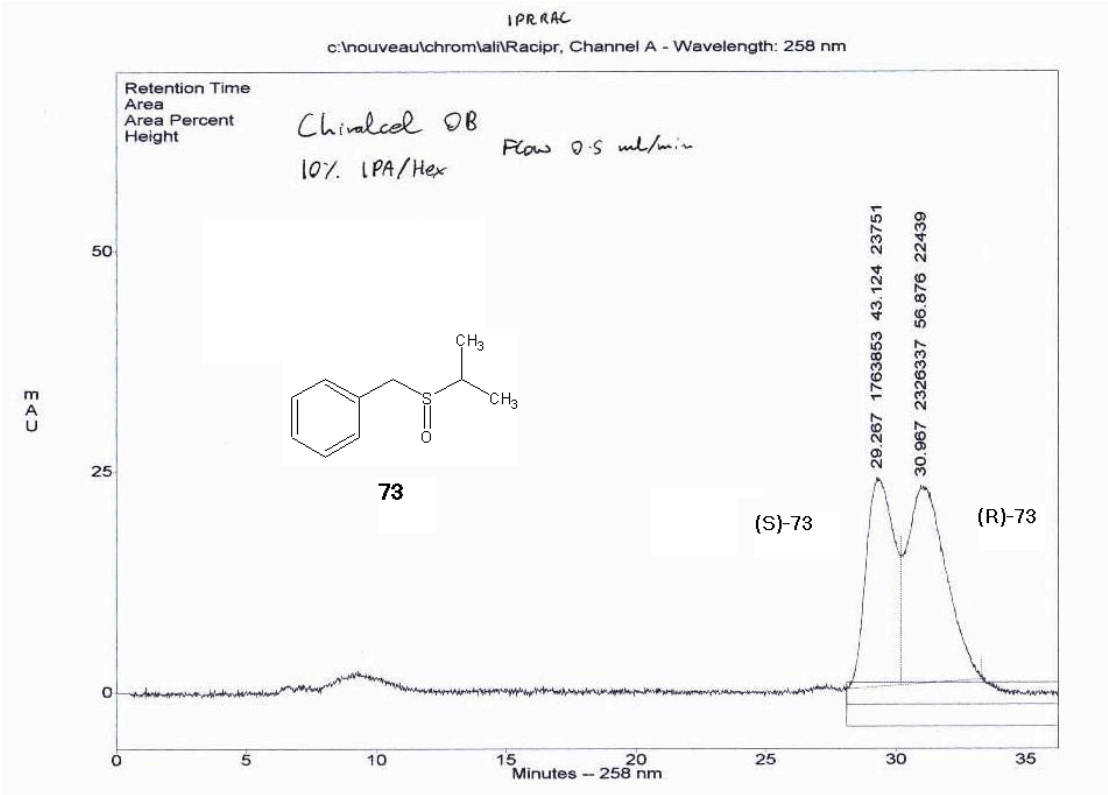

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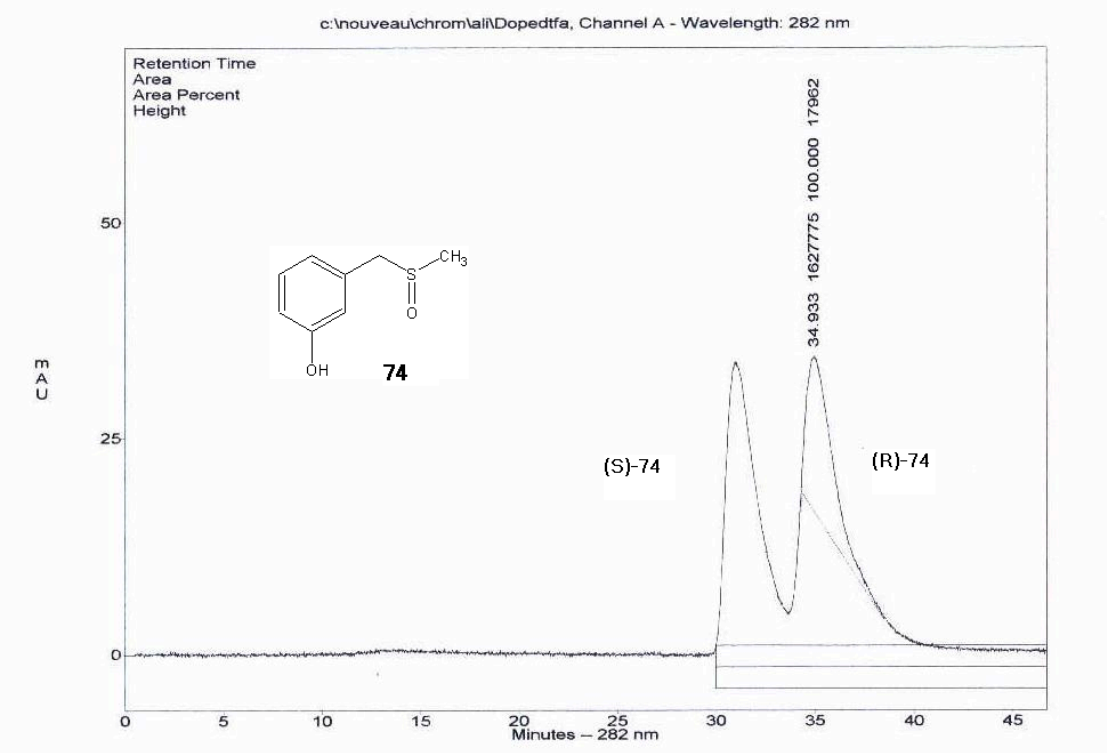

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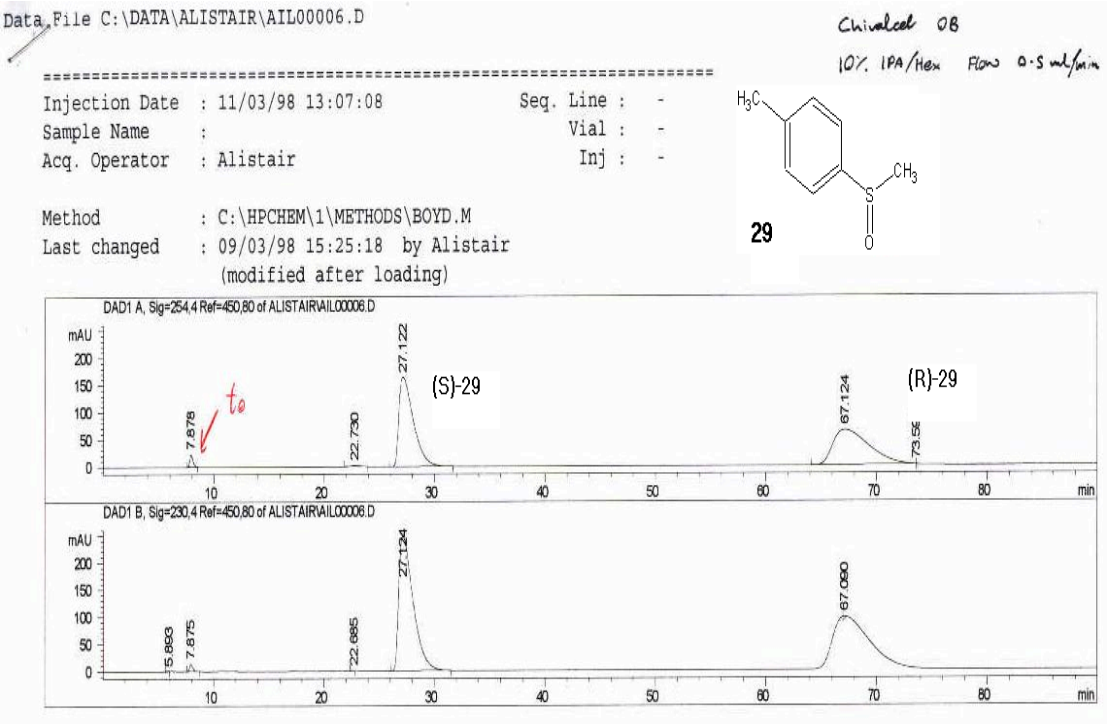

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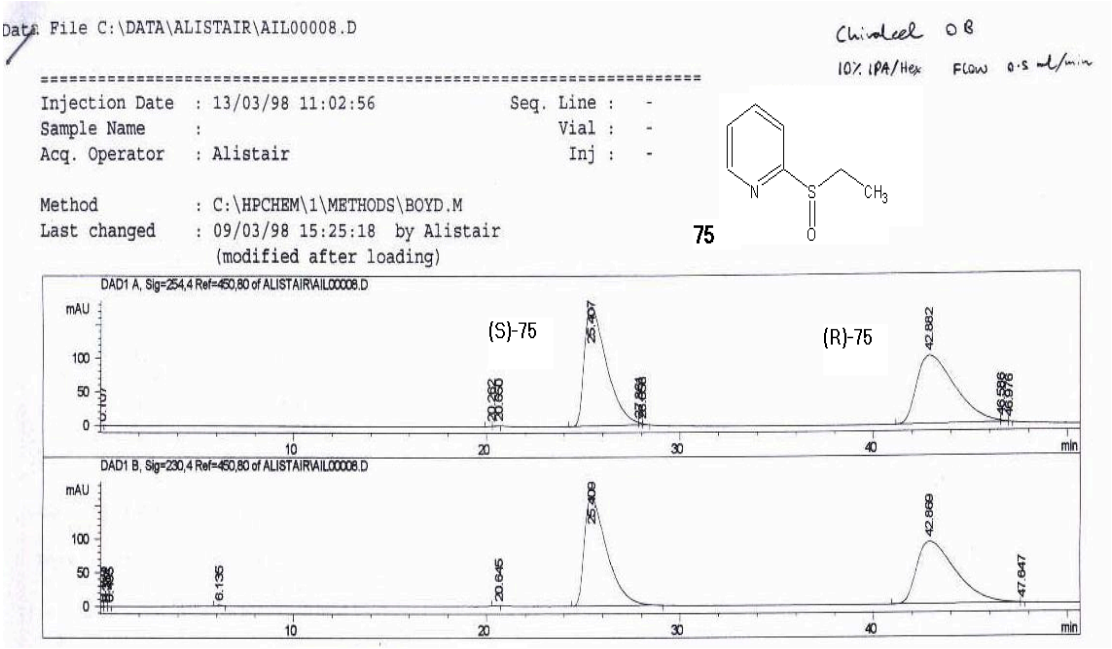

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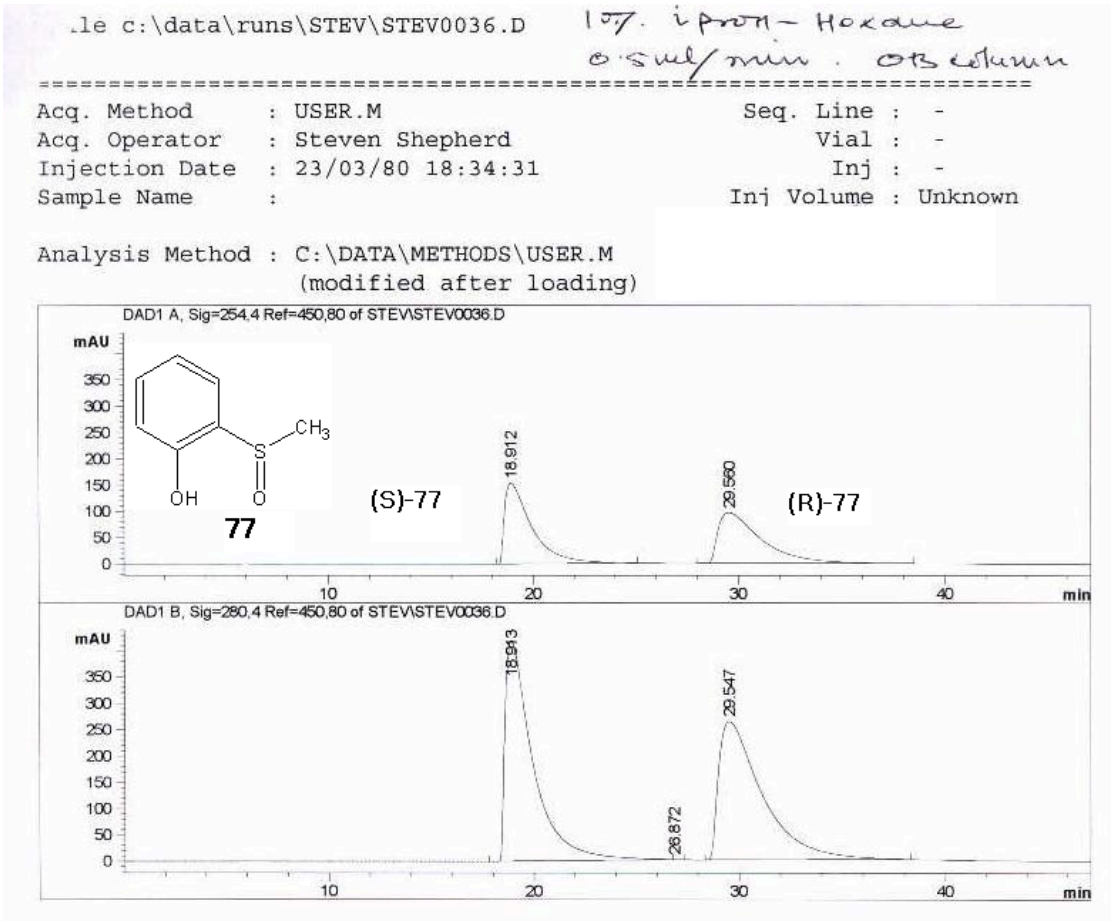

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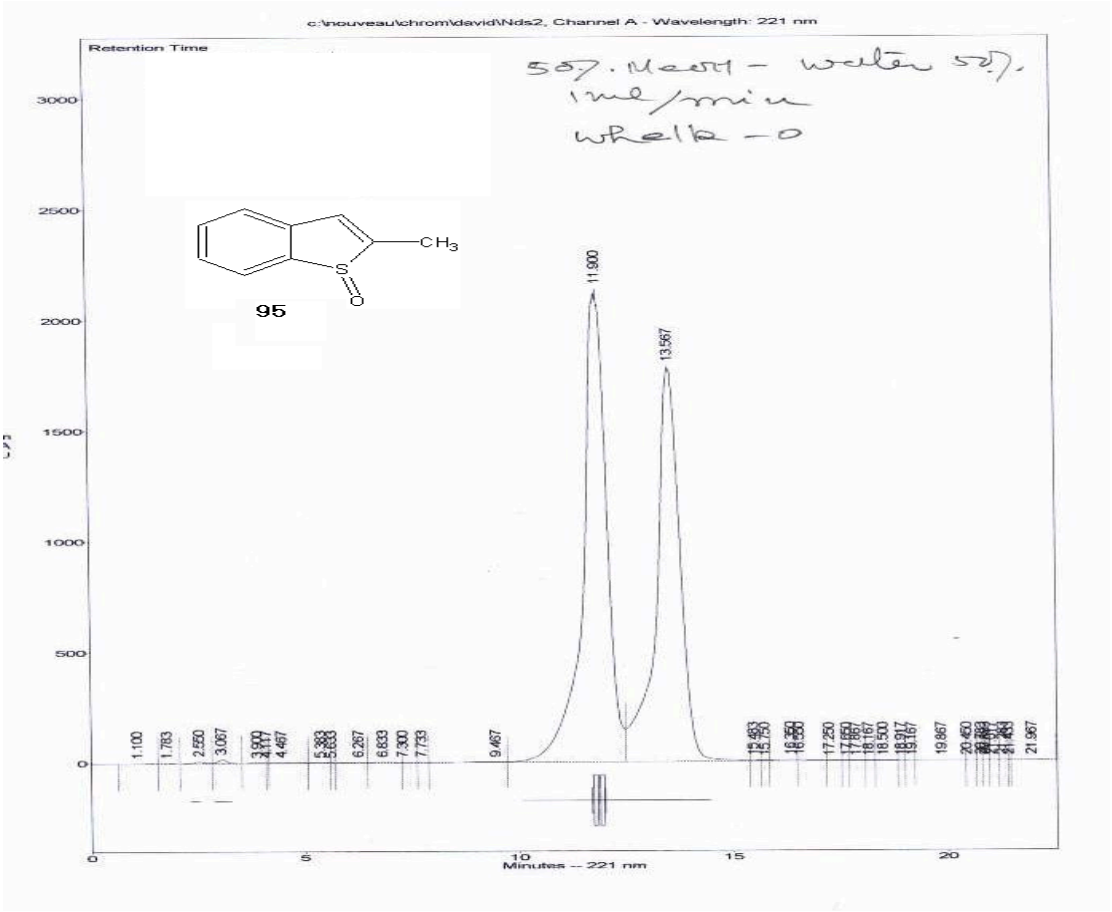

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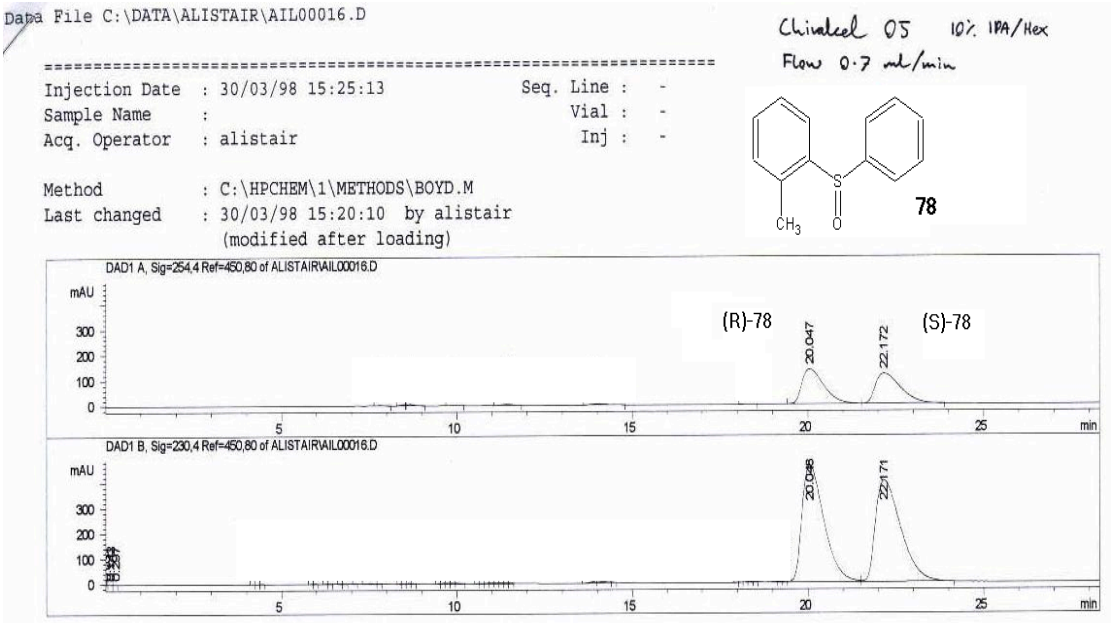

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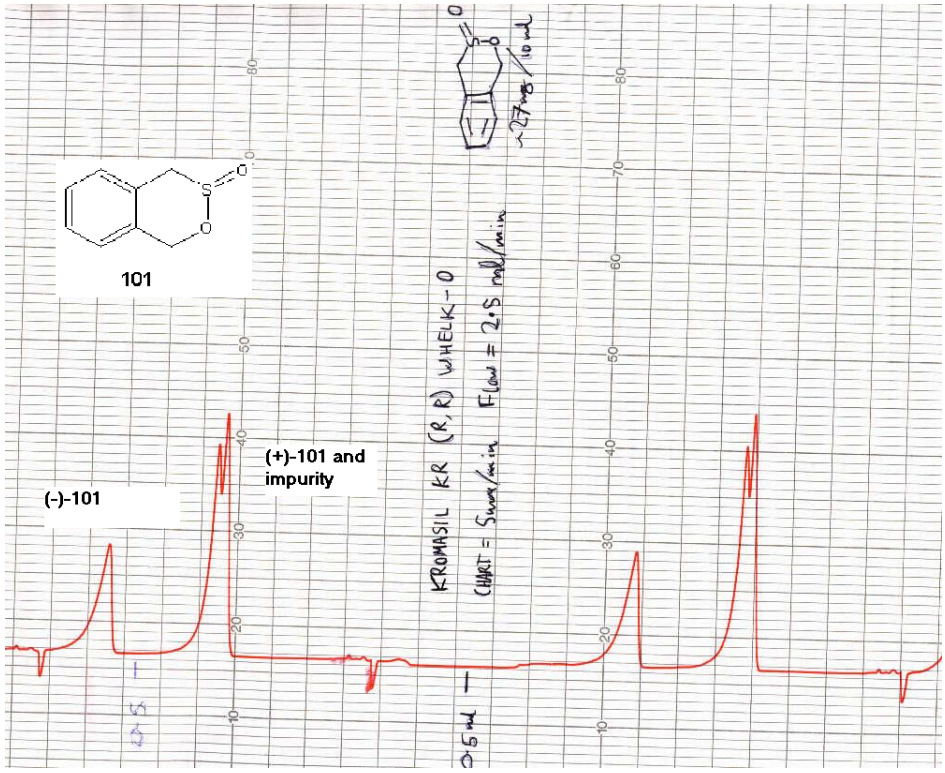

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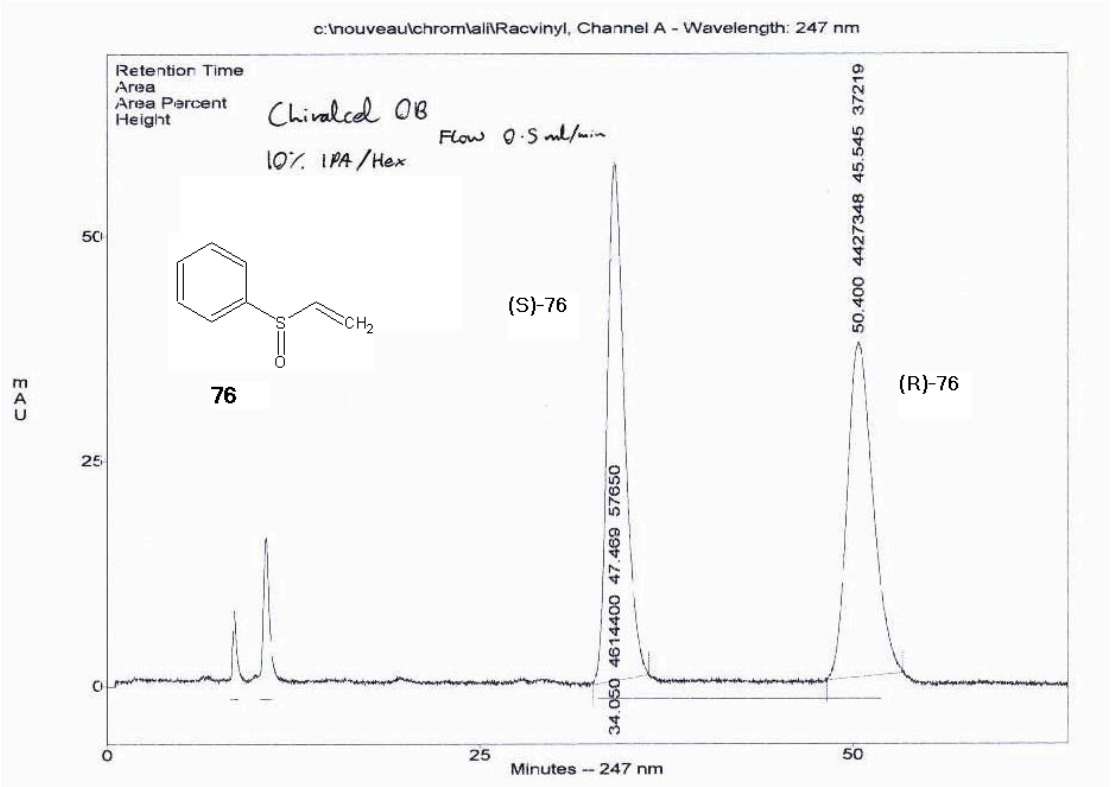


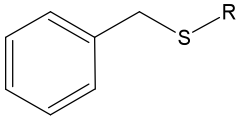
Chapter 3. Chemoenzymatic asymmetric synthesis of chiral sulfoxides.

Chapter 2 demonstrated the potential for the synthesis of chiral sulfoxides by kinetic resolution of sulfoxide racemates using DMSO reductases. This approach has proved to be particularly useful for functionalised sulfoxides, which are difficult to synthesise by the dioxygenase-catalysed oxidation route. Functionalised sulfoxides have also been shown to catalyse enantioselective diethylzinc alkylations of benzaldehyde **101**. A major objective of this chapter will be to show that alternative chemoenzymatic methods are available for synthesising ligands that could catalyse this type of reaction. A further objective will be to synthesise new chiral sulfoxides using dioxygenases.

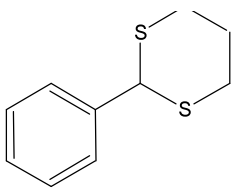
3.1. Asymmetric synthesis of chiral dialkyl sulfoxides using dioxygenases.

Dioxygenases have been found to catalyse the enantioselective sulfoxidation of a range of sulfides, generally where the sulfur atom is α- to an aromatic ring (alkylaryl and diaryl sulfides). Few examples have been reported of dialkyl sulfides undergoing dioxygenase-mediated sulfoxidations. One study into biological oxidation of dialkyl sulfides, by Colonna *et al*,[107] has evaluated the use of purified cyclohexanone monooxygenase (CMO) and chloroperoxidase (CPO) enzymes in the synthesis of enantioenriched chiral dialkyl sulfoxides. These methods give a range of chiral dialkyl sulfoxides with high enantiopurity values and enantiocomplementarity between enzymes, although there are restrictions on the size of substrates, showing decreased yields and enantiopurity with increasing size and chain length. Perhaps dioxygenase-mediated metabolism can provide alternatives for certain substrates.

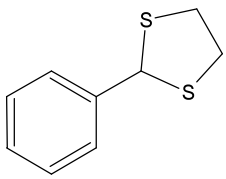
To investigate whether dioxygenase-catalysed sulfoxidations can occur on dialkyl sulfides, a range of alkyl benzyl sulfides **81**, **87-89**, 2-phenyl-1,3-dithiane **110** and 2-phenyl-1,3-dithiolane **111** were studied.



R = Me **81**
R = Et **87**
R = Pr **88**
R = Pr^i **89**



2-Phenyl-1,3-dithiane
110



2-Phenyl-1,3-dithiolane
111

These substrates were selected as the benzene ring may satisfy the requirements for binding to the active site, and may facilitate sulfoxidation. The chromophore also assists in the identification and purification of any possible bioproducts.

The selected organosulfur substrates (alkylbenzyl sulfides and thioacetals) have also previously been metabolised by a range of microorganisms in some cases giving mixtures of *cis* and *trans* sulfoxides (**Table 3.1.**). From **Table 3.1.** it is clear that while the yields are moderate to good, the ee values obtained are low. There are only three examples with ee values > 90% which can be compared with dioxygenase-mediated metabolism of alkylaryl and diaryl sulfides[30,90] where there are many cases of high yields and ee values, with enantiocomplementarity between strains.

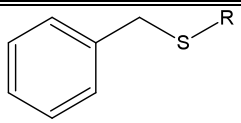
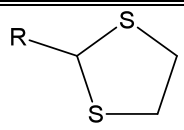
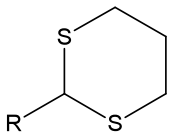
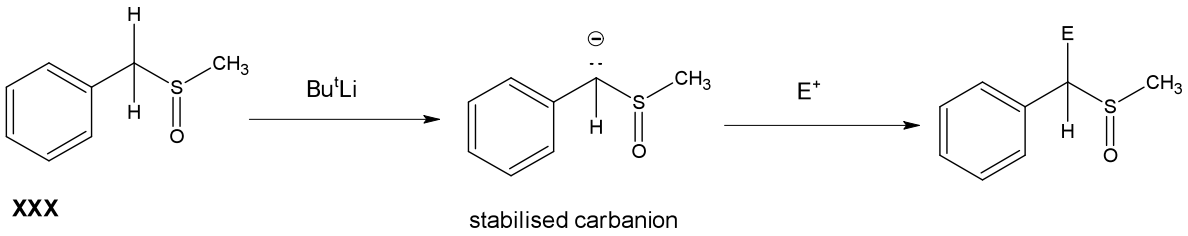
			
Acinetobacter calcoaceticus NCIMB 9871 Ref. **107**, **108**, **100**	**R = Me** 97% yld, 54% ee (*R*)	**R = Me** 90% conv. 95% ee *trans*-(1*R*,2*R*)	**R = Me** 100% conv. 50% ee *trans*-(1*R*,2*R*)
	R = Et 80% yld, 67% ee (*S*)	**R = Ph** 98% conv. 28% ee *trans*-(1*R*,2*R*)	
	R = Pr 90% yld, 96% ee (*S*)		
	R = Pr^{i} 95% yld, 80% ee (*S*)		
Helminthsporium sp. NRRL 4671 Ref. **109**, **110**	**R = Hex** 82% ee (*S*)	**R = Me** 28% yld, 33.5% ee *trans*-(1*S*,2*S*) 2% yld, 36% ee *cis*-(1*S*,2*R*)	
	R = Hept >95% ee (*S*)	**R = Bu^{t}** 20% yld, 72% ee *trans*-(1*S*,2*S*) 2% yld, 35% ee *cis*-(1*S*,2*R*)	
Mortierella isabellina NRRL 1757 (ATCC 42613) Ref. **111**		**R = Me** 20% yld, 32% ee *trans*-(1*R*,2*R*)	

Table 3.1. *Biological sulfoxidation of some benzylalkyl sulfides and thioacetals*.

All of the sulfides **81**, **87-89, 110** and **111** have the same benzyl sulfide moiety (PhCHRSR) in common. Single enantiomer sulfoxides containing this group have potential as chiral auxiliaries in asymmetric synthesis as the benzylic protons are doubly activated by the aromatic ring and the sulfoxide, making them easily derivatised *via* a stabilised carbanion (**Scheme 3.1.**).



XXX stabilised carbanion

Scheme 3.1. *Derivatisation of a benzyl sulfoxide*.

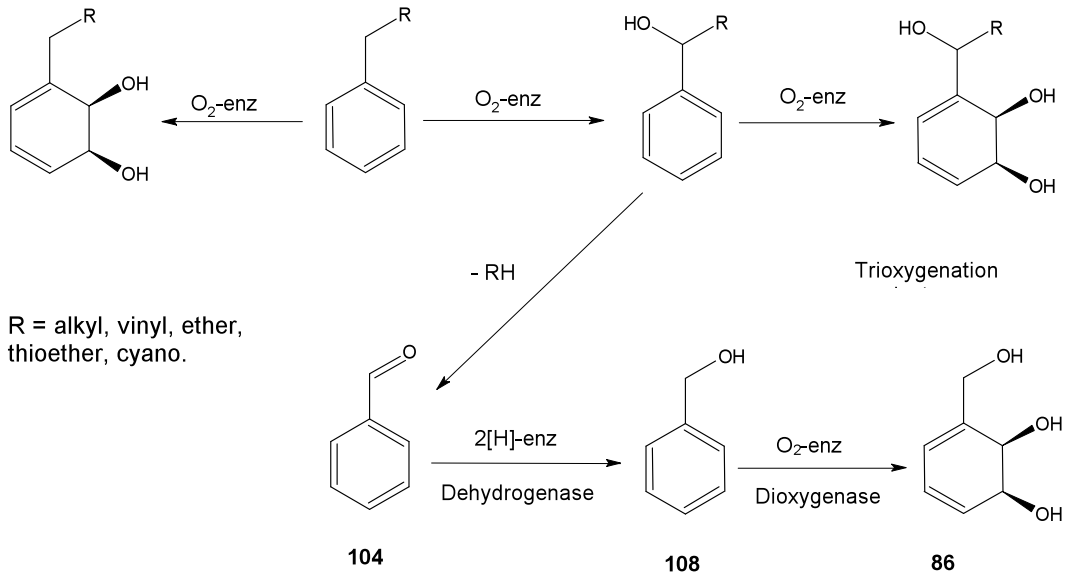
3.2. Dioxygenase-mediated oxygen incorporation.

In the literature there have been cases of dioxygenases catalysing mono-, di-, tri- and even tetra-oxygenation of substrates[24,30,112] Asymmetric monooxygenation of a range of alkylaryl sulfides to sulfoxides having variable ee values and configurations were found using different dioxygenases present in whole-cell bacterial cultures. Dioxygenation of a wide range of aromatic substrates has resulted in > 350 known *cis*-dihydrodiols.[24]

In relation to organosulfur compounds, dioxygenases can catalyse mono- and di-oxygenation processes with the incorporation of either one or both oxygen atoms from molecular dioxygen into the substrate. During *cis*-dihydrodiol production both oxygen atoms are incorporated and only one oxygen atom incorporated in the case of sulfoxidation, with the other atom being converted to a molecule of water. Literature examples of tri- and tetra-oxygenation are discussed below.

3.2.1. Trioxygenation of substituted benzene substrates.

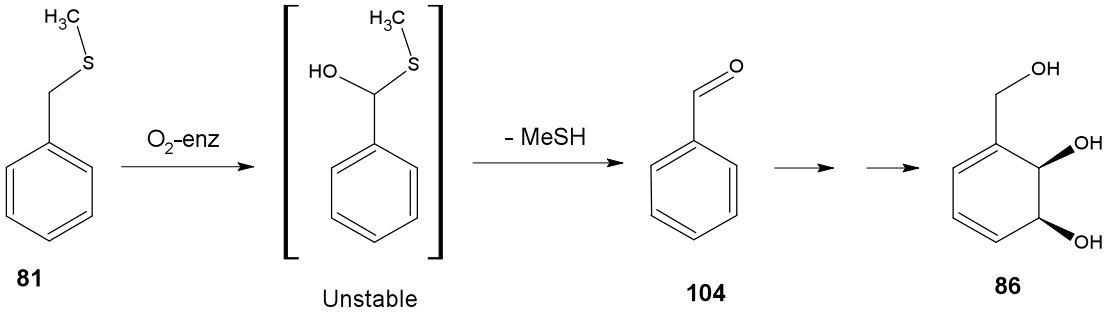
Examples of trioxygenation of substrates have been reported in the literature where substituted benzenes were biotransformed by *P. putida* UV4 to triols[113] (**Scheme 3.2**).



Scheme 3.2. *Trioxygenation of substututed benzenes*.

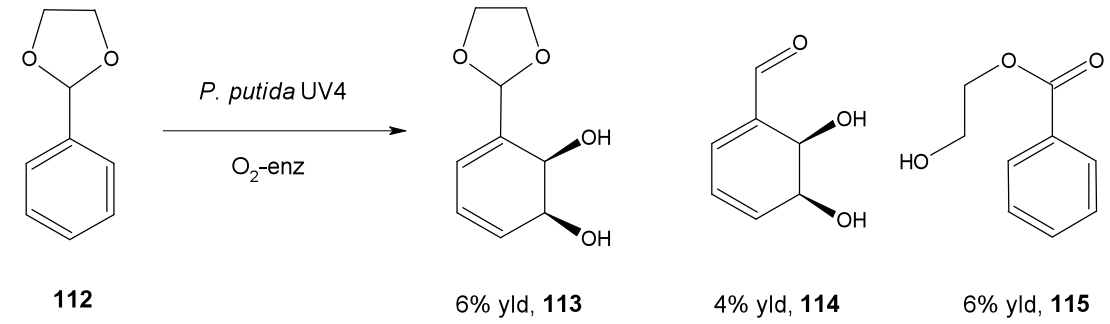
In this study a range of benzylic substrates having, for example, alkyl, vinyl and ether substituents, were metabolised by *P. putida* UV4. In addition to the expected *cis*-dihydrodiol metabolites, triols were also formed due to the α-carbon being hydroxylated. The reaction sequence has been found to be due to both dihydroxylation and monohydroxylation steps followed by either of two pathways. 1) Dihydroxylation of the benzylic alcohol metabolite or 2) cleavage of the acetal side chain giving benzaldehyde **104**. Reduction of the aldehyde **104** to benzyl alcohol **108** was catalysed by an alcohol dehydrogenase enzyme present in *P. putida* UV4. Benzyl alcohol **108** was finally dihydroxylated to yield triol **86**. These two metabolic sequences were confirmed by addition of a range of parent *cis*-dihydrodiol metabolites as potential substrates, without observing any triol metabolites.

Benzylmethyl sulfide **81** has previously been metabolised by *P. putida* UV4 yielding trio **86** as a minor product and an equivalent yield of sulfoxide.[113] This can be attributed to benzylic hydroxylation leading to an unstable hemithioacetal. It was assumed to hydrolyse to benzaldehyde **104**, which has previously been converted to triol **86** by *P. putida* UV4.[104] These results suggest that in *P .putida* UV4 an alcohol dehydrogenase enzyme is present, in addition to a dioxygenase responsible for the *cis*-dihydroxylation to triol **86** (**Scheme 3.3.**).



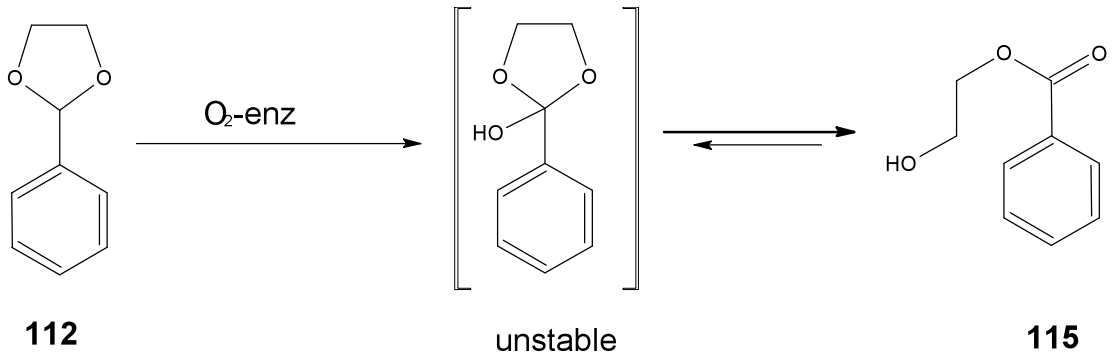
Scheme 3.3. *Trioxygenation of benzylmethyl sulfide* ***81****.*

A similar example of heteroatom / alkyl cleavage of the benzene substituent, through benzylic hydroxylation, has been observed before in a biotransformation carried out with *P. putida* UV4 on 2-phenyl-1,3-dioxane **112** as substrate. This substrate was first metabolised using *P. putida* F39/D (TDO source) by Wackett *et al*[115] yielding only the *cis*-dihydrodiol **113.** When carried out in these laboratories[116] with *P. putida* UV4, three bioproducts were observed, *i.e.* the *cis*-dihydrodiols **113**, **114** and the ester **115** (**Scheme 3.4.**)



Scheme 3.4. *Biotransfomation of acetal* ***112****.*

The bioproduct **115**, although not a trioxygenated product, is thought to be produced by benzylic hydroxylation of the acetal **112** to yield an unstable product which decomposes by cleavage of a C-O bond giving the ester **115** (**Scheme 3.5.**).



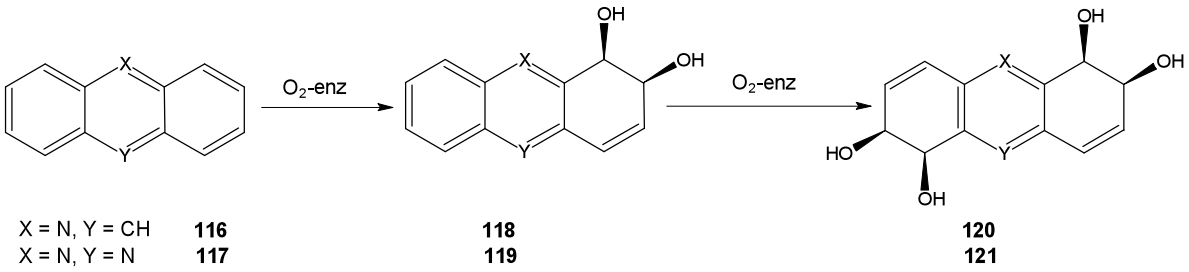
Scheme 3.5. *Benzylic hydroxylation of acetal substrate **112**.*

Metabolite **114** is thought to be a decomposition product of *cis*-dihydrodiol **113**. This is due to the fact that when benzaldehyde **104** is metabolised by *P. putida* UV4 the triol **86** is produced, rather than the bioproduct **114**.

The dioxygenase-catalysed benzylic hydroxylation can perhaps be considered to be analogous to the heteroatom oxidation found when substrates such as phenylmethyl sulfide **23** is metabolised by dioxygenases, giving sulfoxide **24**. This could be attributed to a preferred oxidation at positions α- to an aromatic ring or other conjugated systems. This regioselective benzylic hydroxylation may explain why there is little sulfoxide produced with the dioxygenase-mediated metabolism of benzylmethyl sulfide **81**,[113] despite sulfur being much easier to oxidise than an alkyl chain or an aromatic ring.

3.2.2. Tetraoxygenation of substrates.

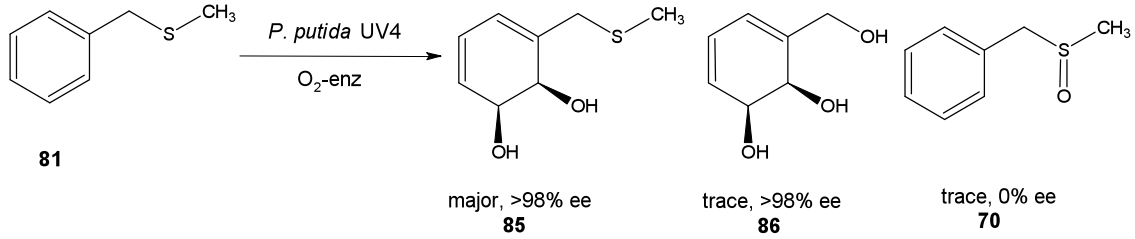
The first examples of dioxygenase-catalysed tetraoxygenation of substrates have recently been reported in the literature.[112] The production of tetraol derivatives of the polycyclic arenes acridine **116** and phenazine **117** was observed in cultures of *Sphingomonas yanoikuyae* B8/36 in these laboratories (**Scheme 3.6.**). The reaction sequence in these cases involving two consecutive dihydroxylation steps. The initially formed *cis*-dihydrodiols **118** and **119** were found to undergo a second dihydroxylation step leading to the corresponding *bis*-*cis*-dihydrodiol (*cis*-tetraols) products **120** and **121**.



Scheme 3.6. *Tetraoxygenation of substrates* ***116*** *and* ***117****.*

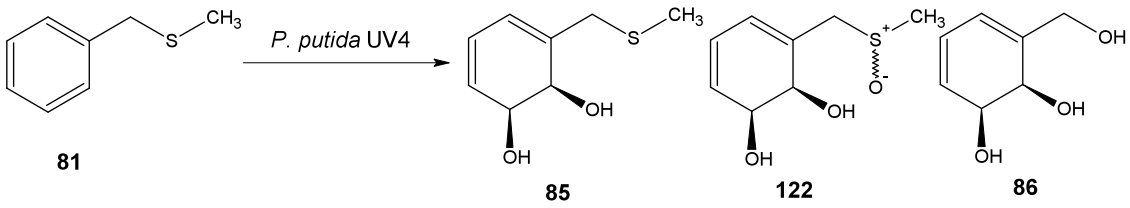
3.3. Metabolism of alkylbenzyl sulfides 81, 87-89 with *P. putida* UV4.

The metabolism of benzylmethyl sulfide **81** by *P. putida* UV4 was previously reported[113] yielding the *cis*-dihydrodiol **85** as the major product, from a dioxygenation. Traces of sulfoxide **70** and triol **86** were obtained from monooxygenation and trioxygenation processes. The diol **85** and triol **86** were found to be enantiomerically pure, but the sulfoxide **70** was racemic (**Scheme 3.7.**).



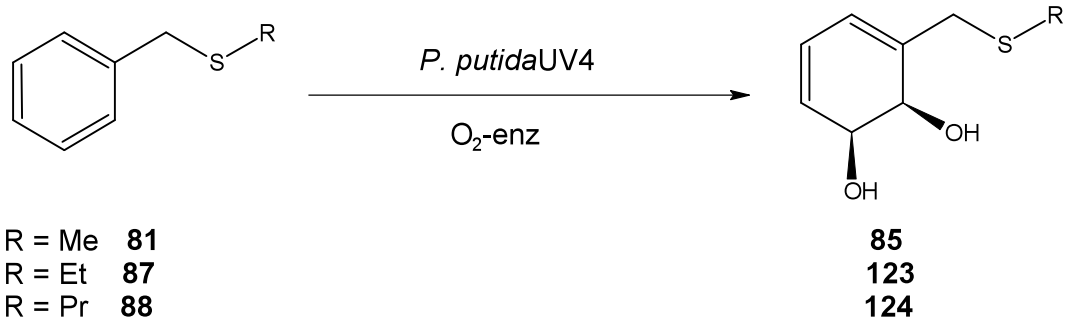
Scheme 3.7.*Reported biotransformation of benzylmethyl sulfide* ***81****.*

When repeated during the current research programme, results were slightly different, with the formation of a new diol sulfoxide trioxygenation product **122** of unknown configuration and ee value, and the absence of any sulfoxide **70** (**Scheme 3.8.**).



Scheme 3.8. *Biotransformation of benzylmethyl sulfide* ***81***.

Metabolism of alkylbenzyl sulfides **87-89**, yielded only the *cis*-dihydrodiols **123** and **124** of unknown configuration and ee values, with benzylisopropyl sulfide **89** giving no bioproduct (**Scheme 3.9.**). Trioxygenation products analogous triol **86** and diol sulfoxide **122** were thus not isolated using *P. putida* UV4 metabolism of benzylalkyl sulfides **88** and **89**.



Scheme 3.9. *Diol bioproducts from the biotransformation of alkylbenzyl sulfides* ***81*** *and* ***87-88***.

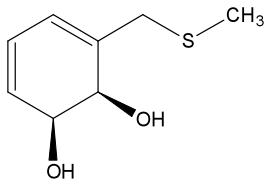
3.3.1. Determination of absolute and relative configurations and enantiopurity values of metabolites from alkylbenzyl sulfides.

3.3.1.1. Enantiopurity determination of the diol sulfides 123 and 124.

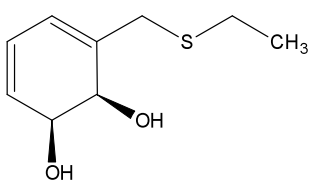
The enantiopurity of the diols from the metabolism of alkylbenzyl sulfides **123** and **124** was determined by the 'chiral boronate method', with the formation of boronate derivatives using (-)- and (±)-2-(methoxyphenylmethyl)benzene boronic acid (MPBBA) **57**, and (-)- and (±)-2-(1-methoxyethyl)benzene boronic acid (MBBA) **54**. No diastereoisomer peak resolution was observed using the (-) MPBBA. Comparison of the NMR spectra obtained with racemic MEBBA did however differentiate between ^{1}H-NMR signals for the diastereoisomers. Enantiopure MEBBA showed no splitting of the ^{1}H-NMR signals due to the presence of a single diastereomer. This observation indicates that the diol sulfides **123** and **124** were enantiopure (> 98% ee), in common with virtually all earlier examples of *cis*-dihydrodiol metabolites of monosubstituted benzene substrates.

3.3.1.2. Absolute configuration determination of the diol sulfides 123 and 124.

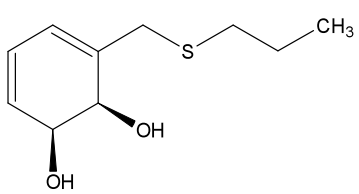
The configurations of the diol sulfides **123** and **124**, with optical rotations of + 69 ($CHCl_3$) and + 73 ($CHCl_3$) respectively, were correlated by CD spectral comparison of enantiopure (+)-*cis*-(1*S*,2*R*)-3-(methylsulfanylmethyl)cyclohexa-3,5-diene-1,2-diol **85**, of known enantiopurity, obtained from the biotransformation of the parent sulfide **81** (**Fig 3.1.**) The CD spectra all had a common positive CD absorption at *ca*: 275nm and weaker positive absorption at *ca*: 230nm consistent with identical (1*S*,2*R*) absolute configurations. This established the metabolites to be (+)-*cis*-(1*S*,2*R*)-3-(ethylsulfanylmethyl)cyclohexa-3,5-diene-1,2-diol **123** and (+)-*cis*-(1*S*,2*R*)-3-(propylsulfanylmethyl)cyclohexa-3,5-diene-1,2-diol **124**.



> 98% **85**



> 98% **123**



> 98% **124**

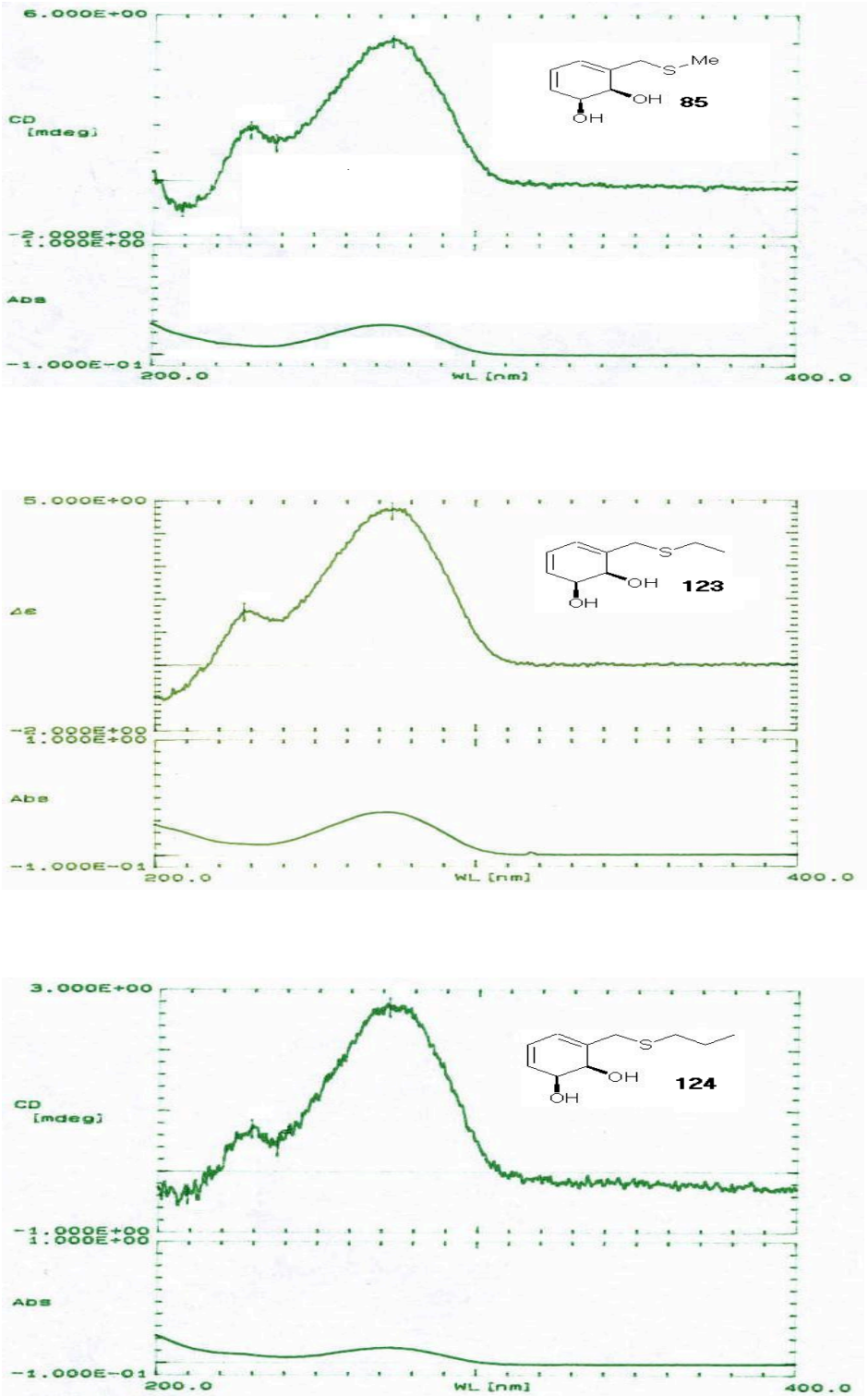


Fig 3.1. *CD correlation of absolute configurations of diol sulfides* ***85***, ***123*** *and* ***124***.

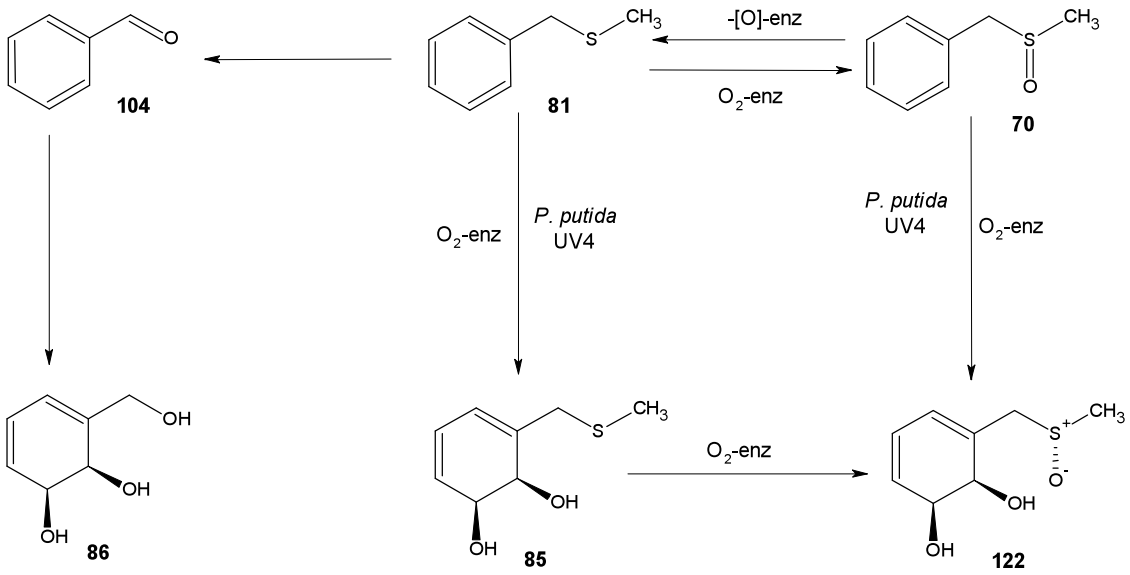
Using the MEBBA boronate method also confirmed the configurations of the diols **123** and **124**, showing δ_{OMe} to be upfield and δ_{Me} to be downfield from the other diastereomers, using the (-)-MEBBA ; the δ values for the (-)-MEBBA derivatives **123** and **124** were 3.152ppm (OMe) and 3.151ppm (OMe) respectively. For the other (+)-MEBBA diastereoisomer, δ values for **123** and **124**, were 3.158ppm (OMe) and 3.158ppm (OMe) respectively. The δ_{Me} values were not reported as they were ill-defined.

3.3.1.3. Determination of absolute configuration and enantiopurity of triol 86 and diol sulfoxide 122.

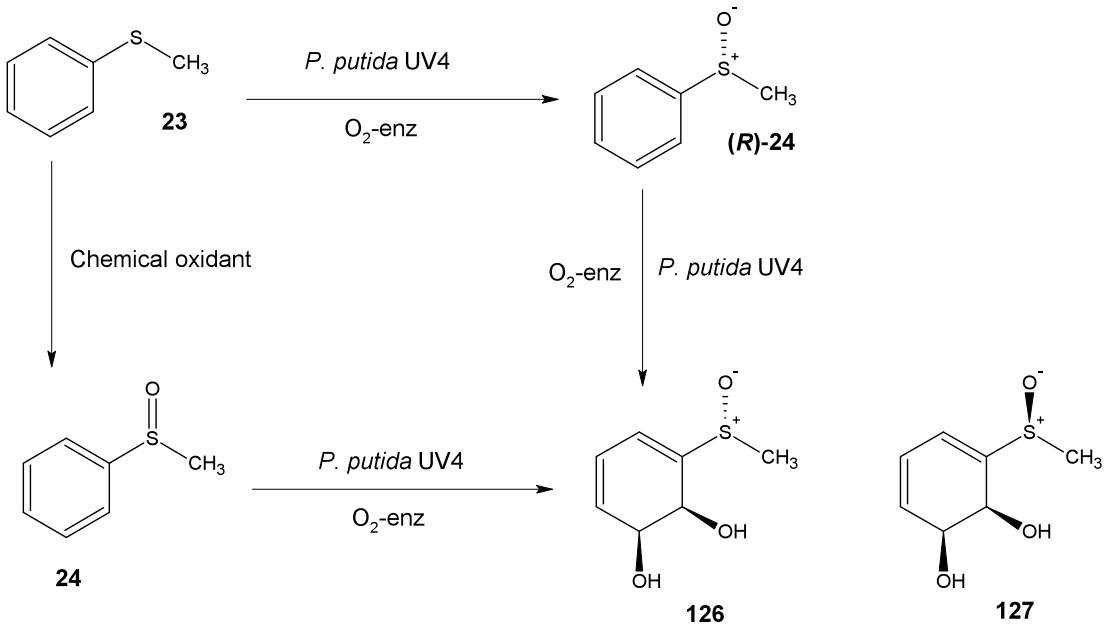
The triol **86** produced from trioxygenation of sulfide **85** was enantiopure, $[\alpha]_D$ + 35 (MeOH), as found in the earlier studies[113]

It is unclear whether the tandem oxidation, to yield diol sulfoxide **122** occurs *via* the sulfoxide **70** (analogous to the sequence found with the triol formation[113]), or the sulfoxidation of the *cis*-dihydrodiol **85** (**Scheme 3.10.**). It was anticipated that reintroduction of dihydrodiol sulfide **85** and racemic sulfoxide **70** as substrates for *P. putida* UV4 could identify the pathway; unfortunately both the substrates afforded diol sulfoxide **122** albeit in trace amounts. In addition to the isolation of **122**, a small quantity of the diol sulfide **85** was isolated, Thus a minor pathway of enzymatic deoxygenation, followed by dihydroxylation was discovered. Recent results, carried out in these laboratories,[117] involving the metabolism of racemic methylphenyl sulfoxide **24** by *P. putida* UV4 showed the formation of diastereoisomeric diol sulfoxide metabolites **126** and **127** in significant yields (**Scheme 3.11.**). This has demonstrated that, in this case (from alkylaryl sulfoxide), the sequence involving sulfoxidation, followed by dihydroxylation (consistant with the triol formation analogy) is the major pathway.

The enantiopurity and configuration of the diol sulfoxide **122** was determined by chemoenzymatic synthesis (DMD oxidation) and separation of the two possible diastereoisomers from the initially formed enantiopure *cis*-dihydrodiol **85**, obtained from the biotransformation of the parent sulfide **81** (**Scheme 3.12.**).



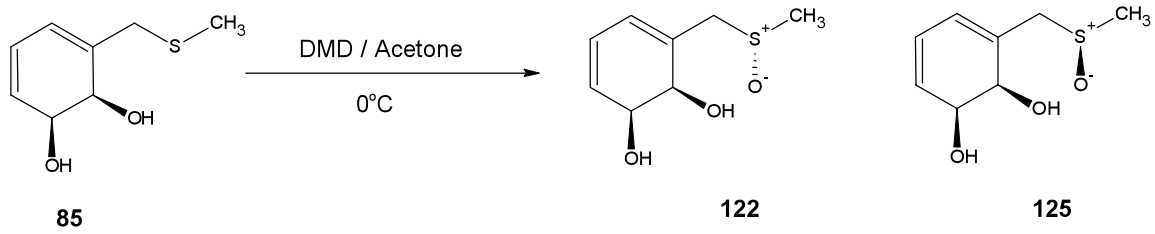
Scheme 3.10. *Dioxygenase-catalysed synthesis of the cis-dihydrodiol sulfide* ***85****, triol* ***86*** *and cis-dihydrodiol sulfoxide* ***122****.*



Scheme 3.11. *Formation of diol sulfoxide diastereoisomers* ***126*** *and* ***127****.*

The diastereoisomers **122** and **125** were separated and purified by PLC. X-ray crystallographic analysis, using the anomalous dispersion method (**Fig 3.2.**), of the less polar diastereoisomer **125** ($[\alpha]_D$ + 56.2, MeOH) showed it to be (+)-*cis*-(1*S*,2*R*)-

3-(*R*-methylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol **125**. The –OH group at C-2 was found to be pseudoaxial due to steric interaction with the C-3 substituent. The C-1 –OH group adopted a pseudoequatorial conformation in the crystalline state. No evidence of intramolecular hydrogen-bonding between the sulfoxide and –OH groups was observed. As the absolute configuration of the less polar diastereoisomer **125** was known, the structure of the more polar bioproduct was deduced to be (+)-*cis*-(1*S*,2*R*)-3-(*S*-methylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol **122**.



85 **122** **125**

Scheme 3.12. *DMD oxidation of cis-dihydrodiol* ***85***.

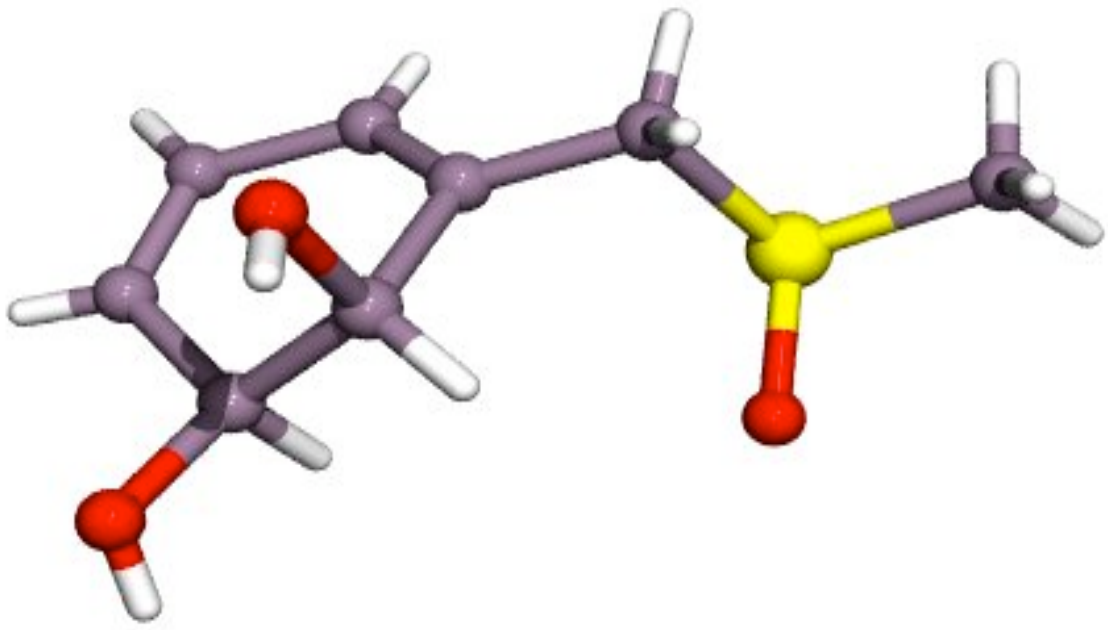


Fig 3.2. *X-ray crystal structure of cis-(1S,2R)-3-(R-methylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol* ***125*** *(Dr J. F. Malone).*

3.4. Metabolism of 2-phenyl-1,3-dithiane 110 and 2-phenyl-1,3-dithiolane 111 with *P. putida* UV4.

The thioacetals 2-phenyl-1,3-dithiane **110** and 2-phenyl-1,3-dithiolane **111** have previously been metabolised on a small scale by *P. putida* UV4 in these laboratories,[90,99] giving a mixture of oxidation products which were not fully identified or characterised. As part of the current programme this biotransformation was revisited but using a larger scale. The products, like those found using benzylmethyl sulfide **81** as substrate, were mixtures of mono-, di- and trioxygenation products.

3.4.1. Assignment of the relative stereochemistry of 1,3-dithiane and 1,3-dithiolane sulfoxides.

NMR analysis has previously been applied to the determination of the relative stereochemistry of *cis*- and *trans*-2-substituted-1,3-dithiane[118] and 1,3-dithiolane sulfoxides.[119,120] *Cis* and *trans* configurations are possible with respect to the sulfoxide oxygen atom and substituent R at C-2. Earlier assignments of the *cis*- or *trans*-geometry of 1,3-dithiane-1-oxides were based on crystallographic and NMR evidence obtained by Carey *et al.*[120] It was observed that $\delta_{2\text{-H}}$ values for *cis* sulfoxides were upfield relative to those for the *trans* sulfoxides. This was due to the 1,3-dithiane and 1,3-dithiolane rings adopting chair and envelope conformations respectively. The anisotropic effect of the sulfoxide group (equatorial) was found to deshield 2-H, which was in a *cis* position relative to the sulfoxide group (axial), causing it to move downfield.

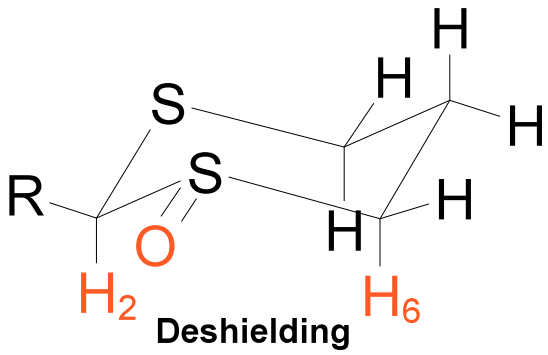
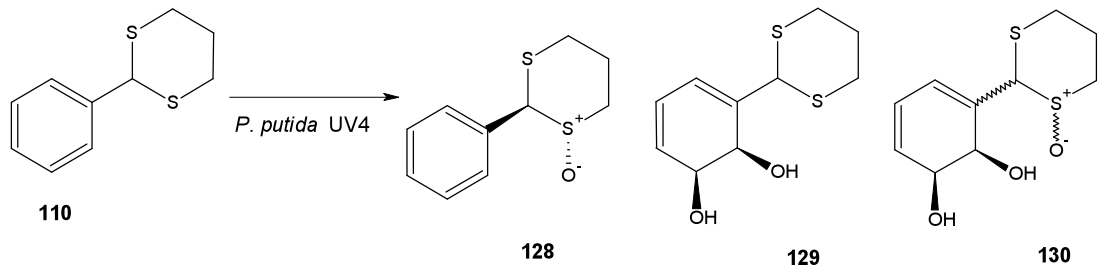


Fig 3.2. *The effect of deshielding by the sulfoxide group on adjacent axial protons in the 1,3-dithiane ring.*

This anisotropic effect is similarly observed for the H-6 proton in the axial opsition, with the *trans*-sulfoxide configuration. In the absence of X-ray crystallography, this method has been of considerable value in determining relative configurations.

3.4.2. Biotransformation of 2-phenyl-1,3-dithiane 110 with *P. putida* UV4.

In a preliminary study by McMurry,[99] biotransformation of 2-phenyl-1,3-dithiane **110** by *P. putida* UV4, gave three bioproducts. The sulfoxide **128** (monooxygenation product) and the diol **129** (dioxygenation product) were stereochemically assigned. However in the case of diol sulfoxide **130** (trioxygenation product), neither the relative nor the absolute configurations were determined (**Scheme 3.13.**).



Scheme 3.13. *Biotransformation of 2-phenyl-1,3-dithiane* ***110****.*

The monooxygenation product, *trans*-(1*S*,2*S*)-2-phenyl-1,3-dithiane-1-oxide **128**, was produced as the minor metabolite (7% yield). The diol sulfide, *cis*-(1*S*,2*R*)-3-(1′,3′-dithian-2′-yl)cyclohexa-3,5-diene-1,2-diol **129**, proved to be the major metabolite (18% yield) and the diol sulfoxide, (1′,3′-dithan-2′-yl-1′-oxy)cyclohexa-3,5-diene-1,2-diol **130**, was also produced in significant amount as a single diastereoisomer (12% yield). These results appeared to be similar to those obtained in the preliminary work carried out earlier in these laboratories,[99] with slight differences in the relative yields. This variation was not unexpected since the proportions of metabolites have been found to change in a rational manner during time-course studies.

3.4.3. Determination of relative and absolute configurations and enantiopurity values of the metabolites 128-130.

3.4.3.1. Enantiopurity determination of sulfoxide 128.

The enantiopurity of *trans*-2-phenyl-1,3-dithiane-1-oxide **128**, obtained earlier from the metabolism of 2-phenyl-1,3-dithiane **110** with *P. putida* UV4, was determined by CSP HPLC (Chiralcel OD column, 50% IPA/hex, 1.0 cm^3/min, α 2.70) and ^{1}H-NMR analysis using 'Pirkle solvent'. Both methods showed the sulfoxide to be a single enantiomer ($[\alpha]_D$ + 94, $CHCl_3$). The enantiopurity of the sulfoxide metabolite formed during the present research programme was determined by comparison with the earlier $[\alpha]_D$ value obtained ($[\alpha]_D$ + 91, $CHCl_3$) In both cases, the dialkyl sulfoxide **128** was assumed to be enantiopure (> 98% ee).

3.4.3.2. Absolute configuration determination of the sulfoxide 128.

The absolute configuration of sulfoxide **128** was previously determined by X-ray crystallography[99] using the anomalous dispersion method (**Fig 3.3.**).

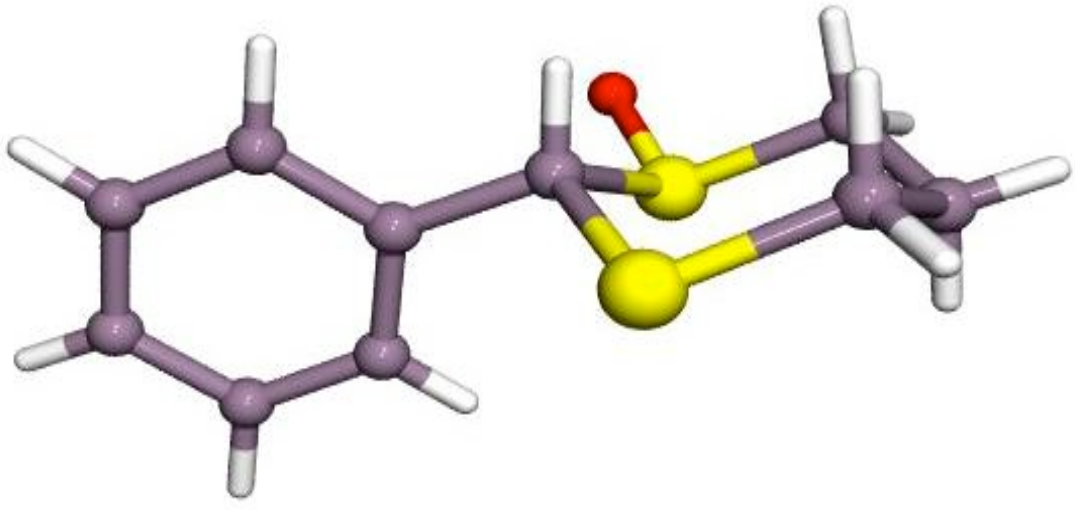
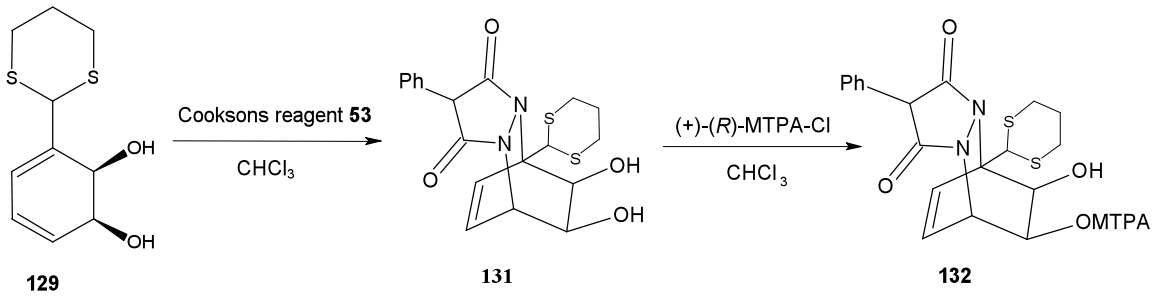


Fig 3.3. *X-ray crystal structure of (+)-trans-(1S,2S)-2-phenyl-1,3-dithiane-1-oxide* ***128*** *(Dr J. F. Malone).*

This provided an unequivocal assignment of the relative (*trans*) and absolute (1*S*,2*S*) configurations of (+)-(1*S*,2*S*)-2-phenyl-1,3-dithiane-1-oxide **128**. In this case the reported NMR method suggested that the relative stereochemistry at the 1,2-position was *cis*. Chemically synthesised samples of the *trans* sulfoxide **128** and the corresponding *cis* isomer showed the 2′-H proton δ_{cis} (4.78 ppm, 300MHz, $CDCl_3$) to be downfield from δ_{trans} (4.57 ppm, 300MHz, $CDCl_3$) This anomaly may be explained by the phenyl group having an additional anisotropic interaction superimposed on that of the sulfoxide group, thus affecting the 2′-H proton.

3.4.3.3. Enantiopurity determination of the diol sulfide 129.

The absolute configuration and enantiopurity of the diol sulfide *cis*-(1*S*,2*R*)-3-(1′,3′-dithan-2′-yl)cyclohexa-3,5-diene-1,2-diol **129** had been determined previously.[99] The ee value was determined by formation of the 'Cookson cycloadduct' **131** using 4-phenyl-1,2,4-triazoline-3,5-dione **53** (Cooksons reagent) as dienophile, followed by formation of a mono (+)-(*R*)-MTPA ester **132** using (+)-(*R*)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid **51** (Moshers acid) (**Scheme 3.14.**). Study of the ^{1}H- and ^{19}F-NMR spectra, for the presence of more that one diastereoisomer, indicated that the *cis*-dihydrodiol **129** was enantiopure. Formation of the mono-MTPA ester rather than the expected diMTPA ester was assumed to be the result of steric hindrance between the proximate 1,3-dithianyl and hydroxyl groups.



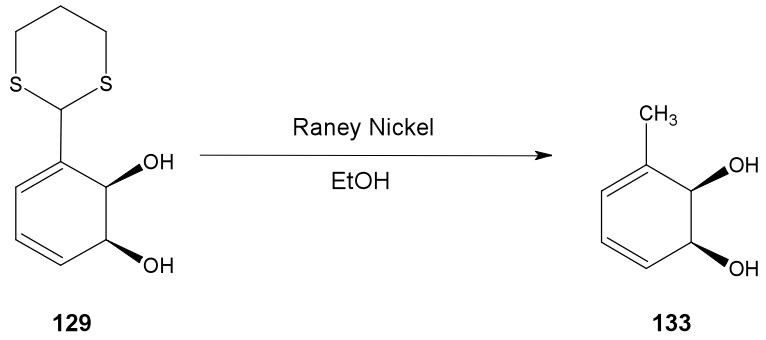
Scheme 3.14. *Determination of enantiopurity of cis-dihydrodiol **127**.*

From a comparison of the specific optical rotation of the parent diol **129**, obtained from the previous preliminary study[99] ($[\alpha]_D$ + 66, $CHCl_3$) with the value obtained from the present research programme ($[\alpha]_D$ + 82, $CHCl_3$), the bioproduct was taken to

be enantiomerically homogeneous. Although the $[\alpha]_D$ values were of different magnitude, the higher value obtained for diol **129** during the later experiment was probably the result of having more metabolite which could be obtained in a higher state of purity.

3.4.3.4. Absolute configuration determination of the diol sulfide 129.

The absolute configuration of the diol sulfide metabolite **129** was previously established[99] by hydrogenolysis of the carbon-sulfur bonds, using Raney nickel. This produced *cis*-(1*S*,2*R*)-3-methylcyclohexa-3,5-diene-1,2-diol **133** (**Scheme 3.15.**), a *cis*-dihydrodiol of known configuration.



129 **133**

Scheme 3.15. *Determination of enantiopurity of cis-dihydrodiol **127**.*

From a comparison of previous ($[\alpha]_D$ values[99] with those obtained during the present research programme, metabolite **129** was again found to be an enantiopure sample of (+)-*cis*-(1*S*,2*R*)-3-(1′,3′-dithian-2′-yl)cyclohexa-3,5-diene-1,2-diol **129**.

3.4.3.5. Absolute configuration and enantiopurity determination of the *cis*-diol sulfoxide 130.

In common with the biotransformation of benzylmethyl sulfide **81** using *P. putida* UV4, a diol sulfoxide diastereoisomer was again isolated from 2-phenyl-1,3-dithiane **110**. The absolute configuration of the diol sulfoxide diastereomer **130** isolated from the biotransformation of the parent dithiane **110**, was determined by oxidation of the diol sulfide **129** with DMD. Two diastereoisomers were produced in the reaction, one of which was spectrally indistinguishable from the diol sulfoxide

metabolite **130**. The $[\alpha]_D$ values of the compound **130** obtained by dioxygenase-catalysed oxidation (+ 49, $CHCl_3$) and by the chemoenzymatic method (+ 47, $CHCl_3$) were comparable. This confirmed that the metabolite was enantiopure and of the same configuration.

The absolute configuration of the diol sulfoxide metabolite **130** was then determined as (+)-*cis*-(1*S*,2*R*)-3-(*trans*-(1′*S*,2′*S*)-1′,3′-dithan-2′-yl-1′-oxy)cyclohexa-3,5-diene-1,2-diol by X-ray crystallography using the anomalous dispersion method (**Fig 3.4.**). The sulfoxide stereocentre of metabolite **130** was found to be of the same configuration (2'*S*) as the sulfoxide metabolite **128**. In the crystalline state the diol sulfoxide **130** adopted a conformation where the sulfoxide oxygen atom and the group at C-2′ were *trans* diequatorial. The –OH group at C-1 was pseudo-equatorial while the –OH group at C-2 was pseudo-axial due to steric interactions between it and the 1,3-dithian–1-oxide group. There was also some evidence of hydrogen bonding between the sulfoxide oxygen atom and–OH group at C-2.

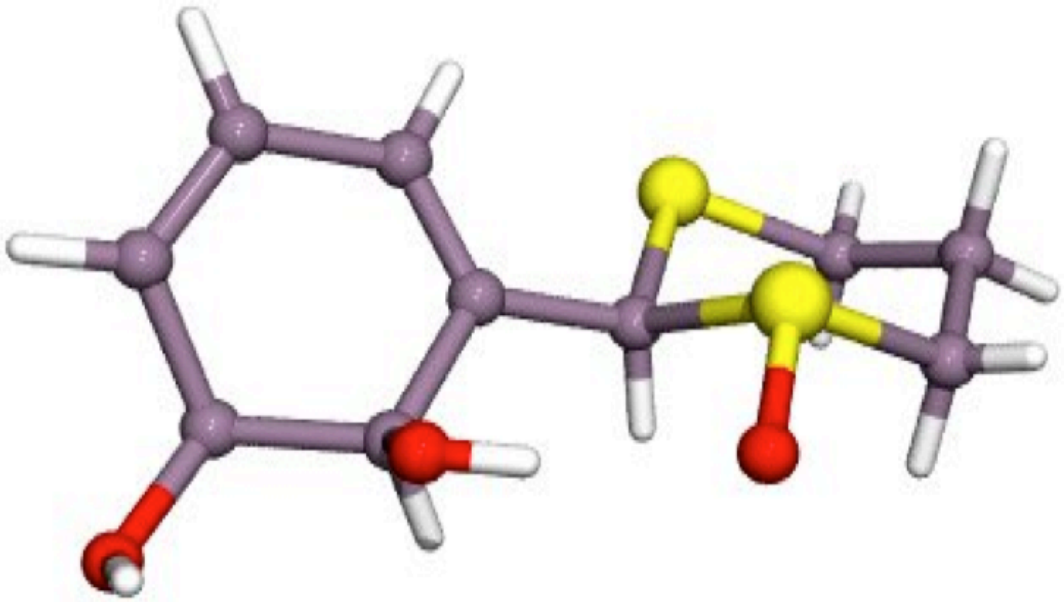
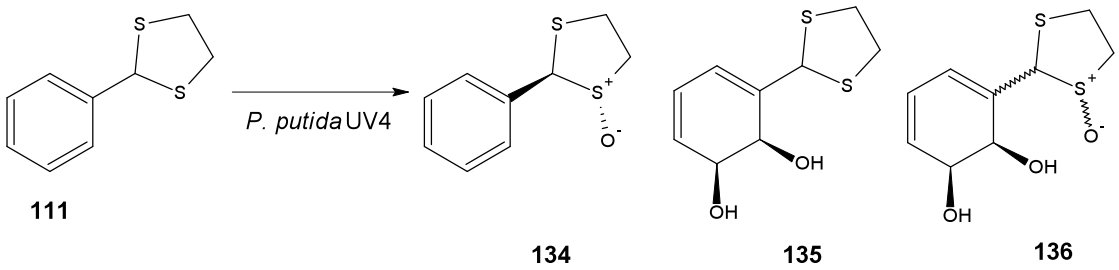


Fig 3.4. *X-ray structure of (+)-cis-(1S,2R)-3-(cis-(1'S,2'S)-1',3'-dithan-2'-yl-1'-oxy)cyclohexa-3,5-diene-1,2-diol* ***130*** *(Dr J. F. Malone).*

3.4.4. Biotransformation of 2-phenyl-1,3-dithiolane 111 with *P. putida* UV4.

A preliminary small-scale biotransformation of 2-phenyl-1,3-dithiolane **111** by *P. putida* UV4,[99] gave two bioproducts, which were identified as the sulfoxide, *trans*-(1*S*,2*S*)-2-phenyl-1,3-dithiolane-1-oxide **134** (monooxygenation product), and the diol *cis*-(1*S*,2*R*)-3-(1′,3′-dithiolan-2′-yl)cyclohexa-3,5-diene-1,2-diol **135** (dioxygenation product). During the present research programme, this experiment was repeated on a larger scale with a longer biotransformation period. Under these conditions a trioxygenation product, the diol sulfoxide 3-(1′,3′-dithiolan-2′-yl-1′-oxy)cyclohexa-3,5-diene-1,2-diol **136**, was found to be the major product. Based upon ^{1}H-NMR analysis of the crude product mixture, which showed mainly metabolite **136**, there was no longer any evidence of sulfoxide **134** (**Scheme 3.16.**) and only traces of diol sulfide **135**.



Scheme 3.16. *Biotransformation of 2-phenyl-1,3-dithiolane **111**.*

The sulfoxide, *trans*-(1*S*,2*S*)-2-phenyl-1,3-dithiolane-1-oxide **134**, was earlier produced as a minor metabolite (3 % yield), while the diol sulfide *cis*-(1*S*,2*R*)-3-(1′,3′-dithiolan-2′-yl)cyclohexa-3,5-diene-1,2-diol **135** was obtained as the major metabolite (15% yield). The diol sulfoxide 3-(1′,3′-dithiolan-2′-yl-1′-oxy)cyclohexa-3,5-diene-1,2-diol **136** was observed after an extended period of biotransformation (8% yield). The diol sulfoxide metabolite **136** was separated from the other metabolites by column chromatrography. The sample obtained was sufficiently pure to allow $[\alpha]_D$ measurements and spectral analysis (NMR, MS and CD). However it proved to be relatively unstable during attempted purification decomposing to give a white solid which was insoluble in common organic solvents. In view of this instability no further work was carried out on diol sulfoxide **136.**

3.4.5. Determination of the absolute configuration and enantiopurity of the metabolites 134-136.

3.4.5.1. Absolute configuration and enantiopurity determination of the sulfoxide 134.

The enantiopurity of *trans*-(1*S*,2*S*)-2-phenyl-1,3-dithiolane-1-oxide **134** was determined during the preliminary small-scale study.[99] CSP HPLC analysis (Chiralcel OD column, α 1.14) showed the sulfoxide bioproduct **134** to have an ee value of 72% ($[\alpha]_D$ – 95, $CHCl_3$). the metabolite obtained during the current work gave a similar $[\alpha]_D$ value ($[\alpha]_D$ – 103, $CHCl_3$). The sample was recrystallised to obtain an enantiopure sample (> 98% ee) with a higher $[\alpha]_D$ value (– 128.9, $CHCl_3$). The ee value of the initial bioproduct was thus determined to be 80%, a slight increase on the earlier small scale biotransformation. From X-ray crystallography, using the anomalous dispersion method, the absolute configuration of the sulfoxide **134** was determined to be (-)-(1*S*,2*S*)-2-phenyl-1,3-dithiolane-1-oxide **134** (**Fig 3.5.**).

From the X-ray structure it is evident that the phenyl ring is *trans* to the sulfoxide oxygen atom which appeared to be in a pseudo-axial conformation. The ^{1}H-NMR evidence was consistent with this configuration. The ^{1}H-NMR spectrum indicated a downfield $\delta_{(2\text{-}H)}$ value of 5.40 ppm compared to $\delta_{(2\text{-}H)}$ 5.29 ppm, associated with a sulfoxide was of *cis* configuration, due to the anisotropic effect of the sulfoxide on the 2-H proton.

3.4.5.2. Enantiopurity determination of the diol sulfide 135.

The enantiopurity of the diol sulfide metabolite *cis*-(1*S*,2*R*)-3-(1′,3′-dithiolan-2′-yl)cyclohexa-3,5-diene-1,2-diol **135** was determined earlier,[99] by formation of the 'Cookson cycloadduct' and then formation of a mono (+)-(*R*)-MTPA ester derivative of the diol sulfide metabolite **135** obtained from the biotransformation of the 1,3-dithiolane **111** (**Scheme 3.17.**).

Comparison of the earlier optical rotation ($[\alpha]_D$ + 60, $CHCl_3$) recorded for diol sulfide **135** with the current value ($[\alpha]_D$ + 33, $CHCl_3$) raised the question of diol sulfide **135** enantiopurity. In order to determine the enantiopurity, chiral boronate derivatives were synthesised using racemic and optically pure 2-(1-methoxyethyl)benzene boronic acid (MBBA) **54** and 2-(methoxyphenylmethyl)benzene boronic acid (MPBBA) **57**.

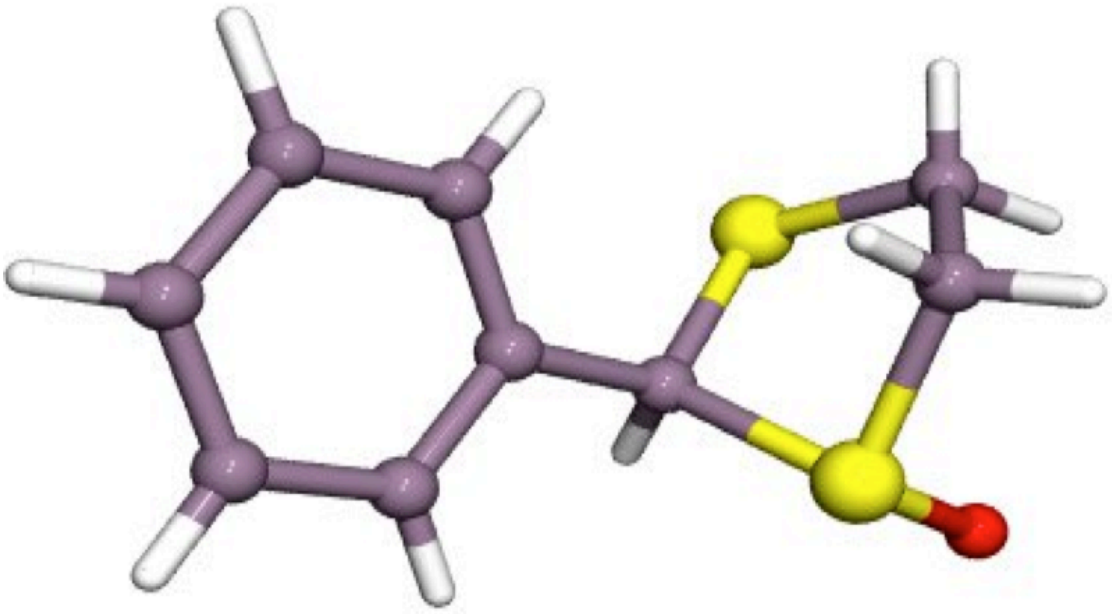
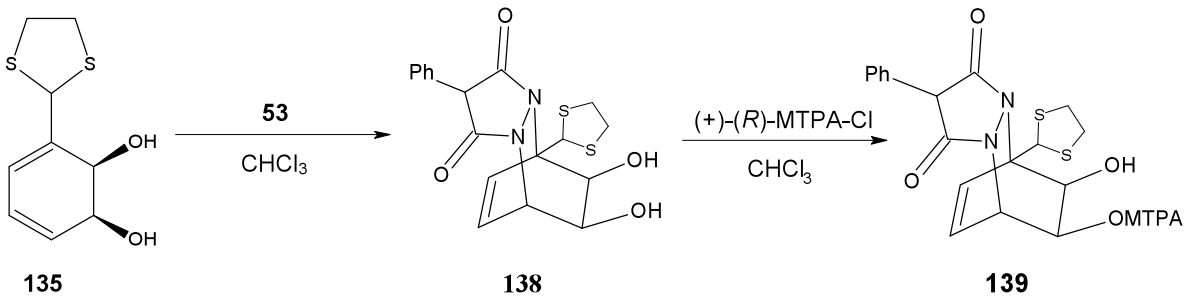


Fig 3.5. *X-ray structure of (-)-trans-(1S,2S)-2-phenyl-1,3-dithiolane-1-oxide* ***134*** *(Dr J. F. Malone).*



135 **138** **139**

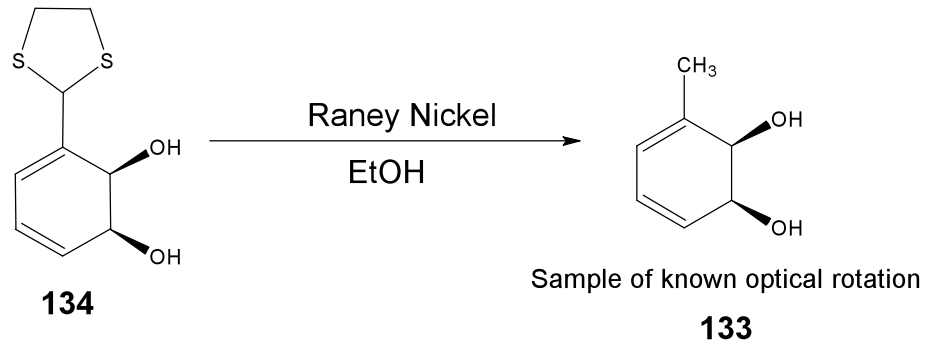
Scheme 3.17. *Determination of enantiopurity of cis-dihydrodiol* ***133***.

The mixture of diastereoisomers formed with MBBA showed an insufficient degree of signal separation in the ^{1}H-NMR spectrum for a reliable ee determination , However by studying the ^{1}H-NMR spectra of the MPBBA diastereoisomer mixtures, it was confirmed that the diol sulfide **133** was enantiopure. It was thus concluded that the earlier $[\alpha]_D$ value obtained from the small-scale experiment was lower than it should have been due to the presence of an impurity.

3.4.5.3. Absolute configuration determination of the diol sulfide 135.

Using the chiral boronate method the absolute configuration of diol **135**, was determined as (1*S*,2*R*) by ^{1}H-NMR analysis which showed δ_{OMe} to be upfield and δ_{Me} to be downfield using the (-)-MPBBA (δ_{OMe} 3.349 ppm) in comparison to the values of the alternative diastereomer obtained using (+)-MPBBA (δ_{OMe} 3.354 ppm).

The absolute configuration of the diol sulfide **135** was confirmed by hydrogenolysis of the carbon-sulfur bonds using Raney nickel to yield *cis*-(1*S*,2*R*)-3-methylcyclohexa-3,5-diene-1,2-diol **133** of known configuration (**Scheme 3.18.**).



134 **133**

Scheme 3.18. *Determination of absolute configuration of cis-dihydrodiol **135**.*

3.4.5.4. Absolute configuration and enantiopurity determination of the diol sulfoxide 136.

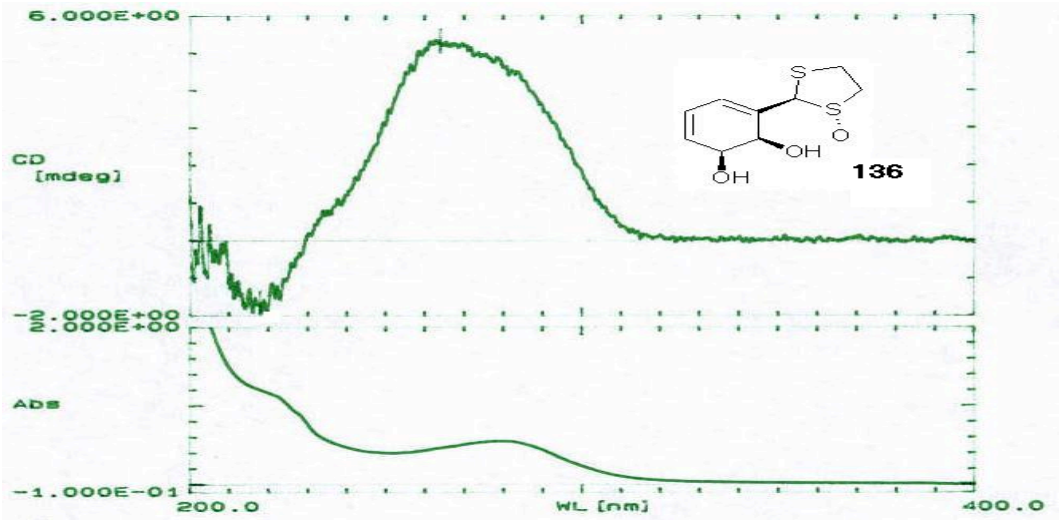
The biotransformation that was left for an extended period, produced mainly the diol sulfoxide diastereoisomer **136**. As yet the ee value and absolute stereochemistrry of metabolite **136** has not been unequivocally determined due to its instability during further purification and attempted formation of derivatives. It is however likely that the c*is*-dihydrodiol **135** was the immediate precursor of diol sulfoxide **136** ($[\alpha]_D$ – 48, $CHCl_3$) and on this basis it was assumed that metabolite **136** was also a single enantiomer. Furthermore, a comparison of the CD spectra of the diol sulfoxide metabolite **130** and the diol sulfoxide metabolite **136** indicates a similarity between their absolute configurations (**Fig 3.6.**). Both CD spectra show negative absorptions at *ca*: 220-230nm followed by strong positive absorption at *ca* : 260-280-nm. While there are similarities between these CD spectra, it does not

provide unequivocal evidence of identical configurations due to the possible influence of further chiral centres.

The ^{1}H-NMR signal for 2′-H in compound **136** which is shifted downfield, (> 5 ppm), suggested a *trans*- configuration between the sulfoxide oxygen atom and the substituent at a C-2′ of the dithiolane ring. Based on the configurations of the other bioproducts obtained from benzylmethyl sulfide **81**, 2-phenyl-1,3-dithiane **110** and 2-phenyl-1,3-dithiolane **111** as substrates for biotransformations with *P. putida* UV4, where any sulfoxides or diol sulfoxides produced had the same absolute configurations at the sulfoxide centres (**Fig 3.7.**), it is a possibility that the sulfoxide centre in metabolite **136** is identical, *i.e.* (-)-*cis*-(1*S*,2*R*)-3-(*trans*-(1′S,2′S)-1′,3′-dithiolan-2′-yl-1′-oxy)cyclohexa-3,5-diene-1,2-diol **136**.

3.5. Conclusion.

Preliminary results obtained from small-scale biotransformations of compounds **81**, **110** and **111**, have now been repeated and confirmed using a larger scale. This has allowed both the structure and stereochemistry of sulfoxides (**128** and **134**), and *cis*-dihydrodiol metabolites (**85**, **129** and **135**), to be fully elucidated. In addition the absolute configurations of the new *cis*-dihydrodiol metabolites **123** and **124** isolated from the biotransformation of substrates **87** and **88** respectively have been determined. A new *cis*-diol sulfoxide metabolite **136**, arising from trioxygenation of substrate **111**, has been identified. From the metabolism studies of sulfides **81**, **110** and **111**, evidence of a tandem oxidation process involving both sulfoxidation and *cis*-dihydroxylation in an unusual sequence has been found.



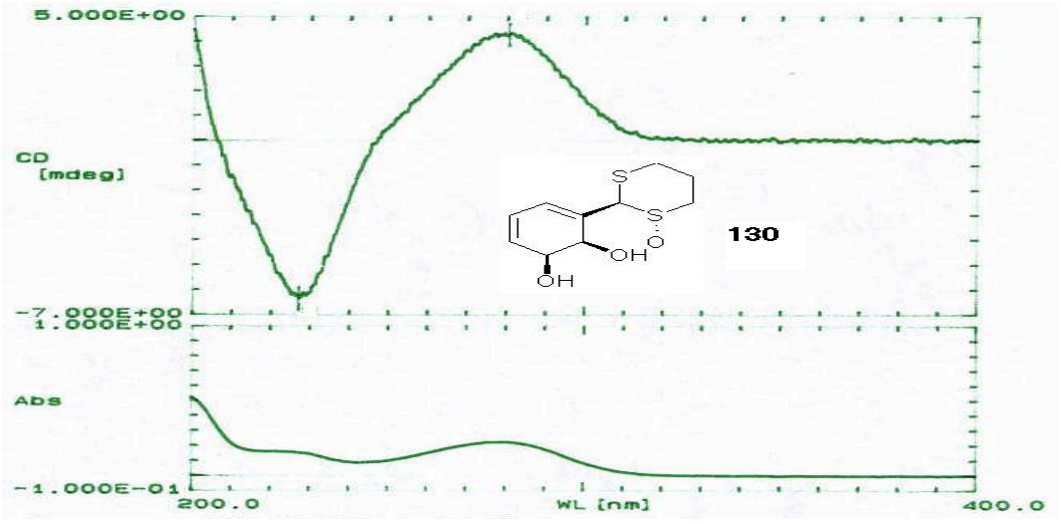


Fig 3.6. *CD spectra of the two diol sufoxide* ***130*** *and* ***136*** *obtained from P. putida UV4.*

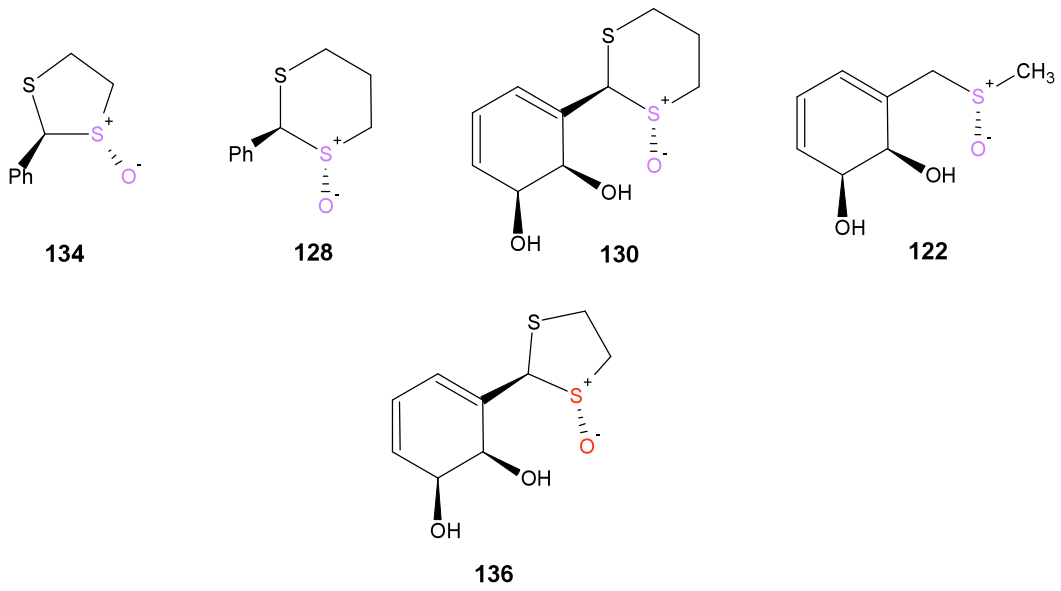


Fig 3.7. *Sulfoxide configurations of bioproducts* ***122***, ***128***, ***130***, ***134*** *and* ***136***.

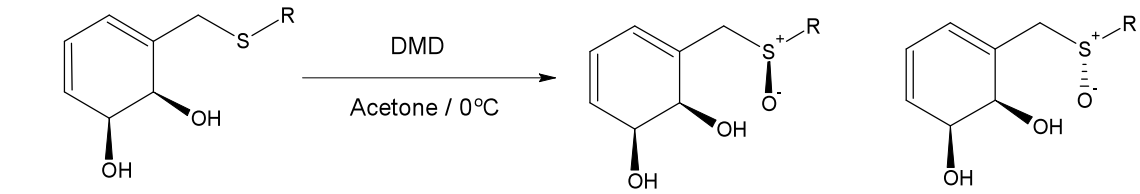
Chapter 4. Synthesis of chiral hydroxylated sulfoxides from *cis*-dihydrodiol bioproducts.

As discussed in Chapter 3, the possibility of obtaining chiral sulfoxides as bioproducts from the dioxygenase-mediated metabolism of a range of alkylbenzyl sulfides (**81**, **87-89**, **110** and **111**) has been examined. A limited degree of success, especially with the thioacetals **110** and **111**, giving sulfoxides **134** and **128** (80% and > 98% ee values respectively) was observed. It was also noted that the dioxygenase enzymes have the ability to produce other types of bioproduct, *e.g. cis*-dihydrodiol sulfides from diooxygenation and *cis*-diol sulfoxides from trioxygenation. It is the aim of this chapter to investigate chemoenzymatic methods for the synthesis of further types of stable functionalised sulfoxides, *i.e.* phenolic sulfoxides, from existing bioproducts, for possible use as chiral synthons.

4.1. Synthesis using bioproducts from the metabolism of alkylbenzyl sulfides 81, 87 and 88, and thioacetal 110 with *P. putida* UV4.

A new method of producing stable functionalised single enantiomer sulfoxides was examined involving oxidation of the diol sulfide metabolites **85**, **123**, **124**, **129** and **135** where two chiral centres already existed. The chirality could in principle be transferred to the exocyclic sulfur centres, by sulfoxidation followed by separation of the resulting diol sulfoxide diastereoisomers. Furthermore, the introduction of a sulfoxide group would be expected to confer increased stability upon the new *cis*-dihydrodiol.

The three diol sulfides **85**, **123** and **124**, obtained from alkylbenzyl sulfide metabolism using *P. putida* UV4, were oxidised using DMD to give mixtures of diastereoisomers in almost equal proportions (**Scheme 4.1.**). The diastereoisomeric pairs **122**, **125** and **140-143** were separated by PLC giving *ca*: 45 % yields for each. The lack of diastereoselectivity during this sulfoxidation suggests that neither of the lone pairs on the sulfur atom were able to exercise stereocontrol due to preferential hydrogen-bonding with the proximate –OH group.



R = Me	**85**	**125**	**122**
R = Et	**123**	**140**	**141**
R = Pr	**124**	**142**	**143**

Scheme 4.1. *Synthesis of diol sulfoxide diastereoisomers **122, 125** and **140-143**.*

Since the *cis*-dihydrodiol precursors **85**, **123** and **124** were enantiopure, the resulting diastereoisomers **122**, **125** and **140-143** were also single enantiomers. Only one of the six diol sulfoxide compounds **122**, **125** or **140-143** was crystalline, which allowed a crystal structure analysis to be undertaken (**Fig 3.2.**). The relative stereochemistry of each member of the remaining diastereoisomeric pairs was suggested by their relative polarity on TLC analysis (the (*S*)-sulfoxide diastereoisomer was consistently found to be more polar *i.e.* lower R_f). These tentative assignments were confirmed by CD spectral comparison (**Fig 4.1.** and **4.2.**), and anchored by the application of X-ray crystallography.

The CD spectra for the less polar (*R*)-sulfoxide diastereoisomers consistently showed weak positive CD absorptions at ~ 225nm,. The CD spectra contained a slight trough, reaching the baseline, followed by a stronger positive absorption at ~ 275nm. The CD spectra for the more polar (*S*)-sulfoxide diastereoisomers consistently showed a negative absorption at ~ 205nm rising to the baseline, followed by a strong negative absorption at ~ 240nm rising to a stronger positive absorption at ~ 270nm. The less polar *cis*-diol sulfoxides were then identified as (+)-*cis*-(1*S*,2*R*)-3-(*R*-methylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol **125**, (+)-*cis*-(1*S*,2*R*)-3-(*R*-ethylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol **140** and (+)-*cis*-(1*S*,2*R*)-3-(*R*-propylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol **142**. The more polar diastereoisomers were found to be (+)-*cis*-(1*S*,2*R*)-3-(*S*-methylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol **122**, (+)-*cis*-(1*S*,2*R*)-3-(*S*-ethylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol **141** and (+)-*cis*-(1*S*,2*R*)-3-(*S*-propylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol **143**.

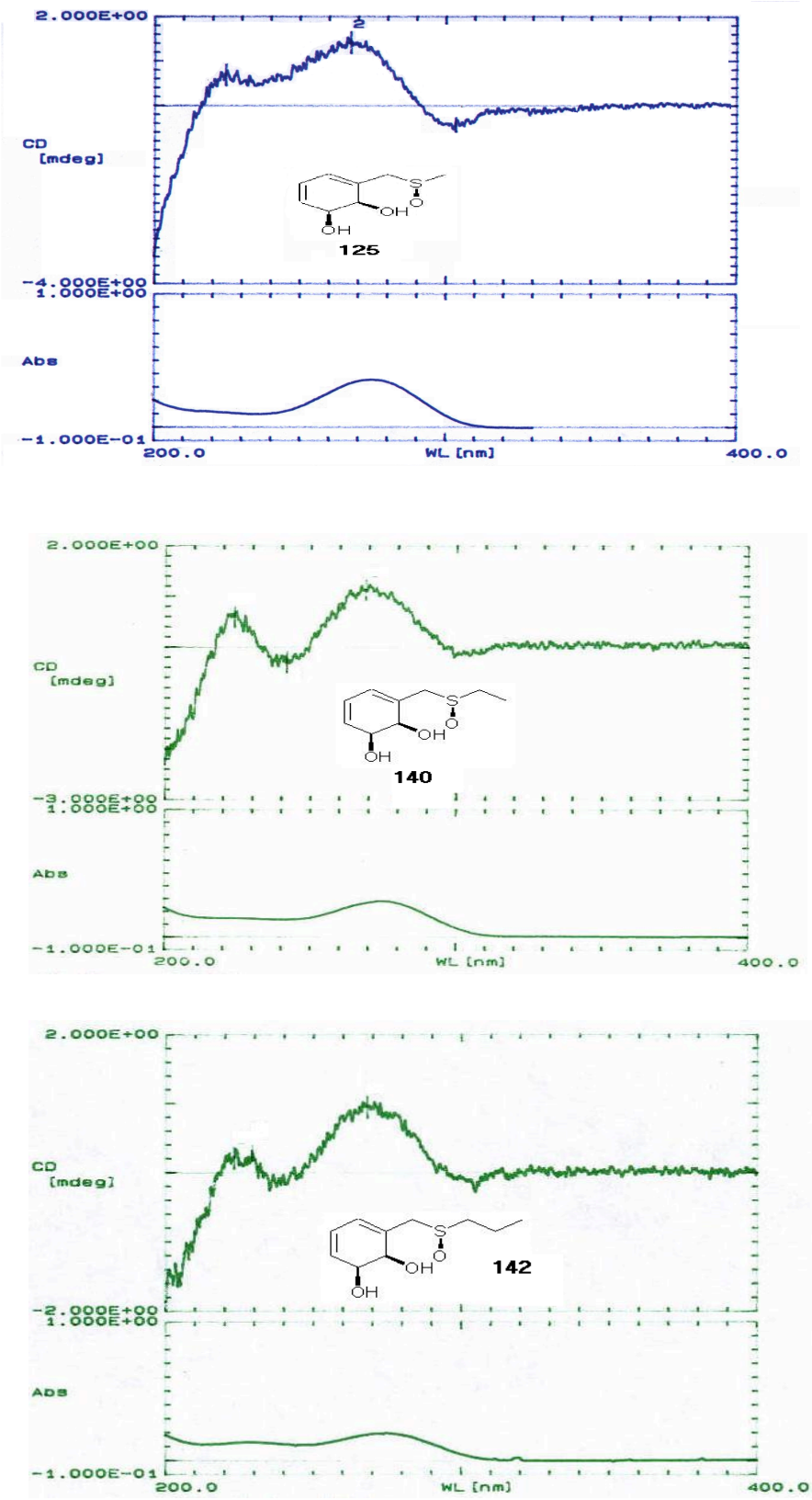


Fig 4.1. *CD correlation of diol sulfoxide diastereoisomers* ***125***, ***140*** *and* ***142***.

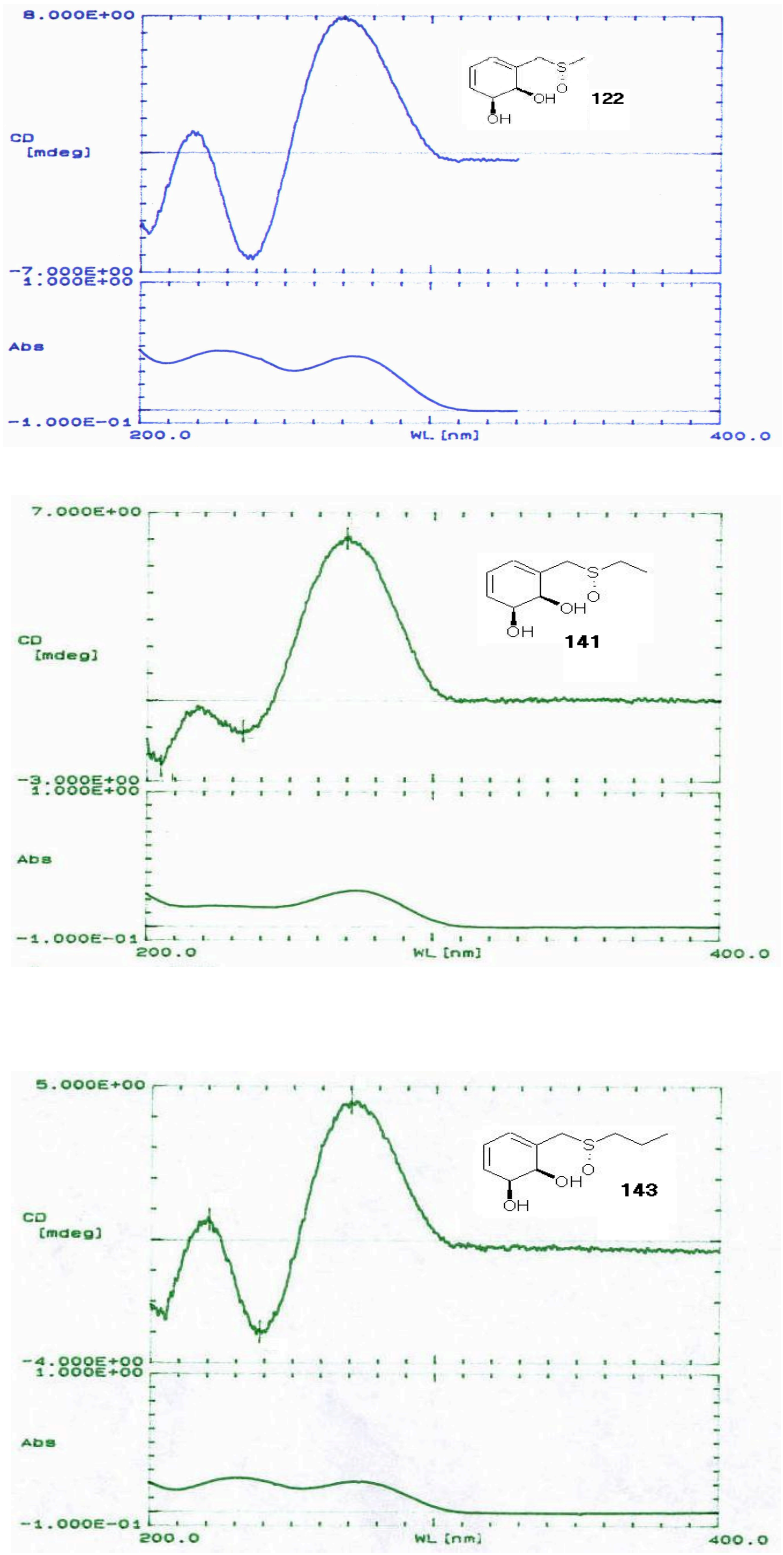
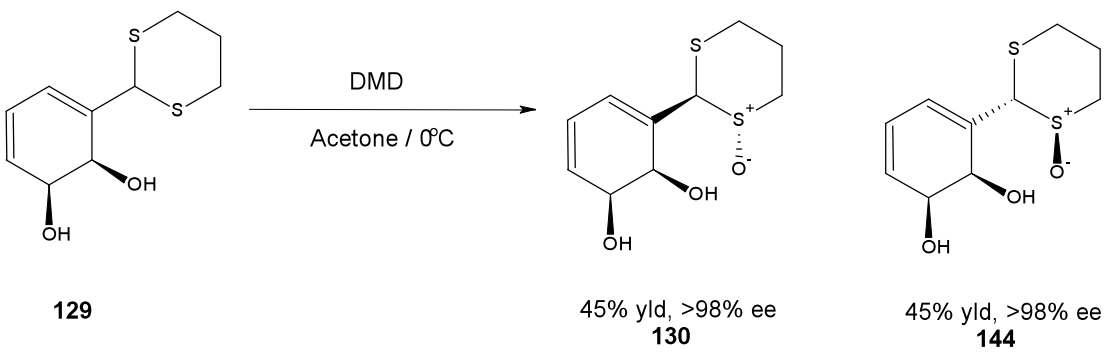


Fig 4.2. *CD correlation of diol sufoxide diastereoisomers* ***122***, ***141*** *and* ***143***.

The X-ray crystal structure (**Fig 3.2.**) also allowed the determination of the stereochemistry of the diol sulfoxide **122**, obtained from the *P. putida* UV4 biotransformation of benzylmethyl sulfide **81**, as discussed in Chapter 3.

Upon DMD oxidation the *cis*-diol sulfide **129**, obtained from the metabolism of 2-phenyl-1,3-dithiane **110** yielded two of the four possible diastereoisomers. The less polar sulfoxidation product was found to be (+)-*cis*-(1*S*,2*R*)-3-(*trans*-(1′*S*,2′*S*)-1′,3′-dithan-2′-yl-1′-oxy)cyclohexa-3,5-diene-1,2-diol **130,** as it was spectrally indistinguishable from the diol sulfoxide metabolite **130** obtained from the biotransformation of the parent 1,3-dithiane **110**. The absolute configuration of compound **130** had been determined by X-ray crystallography (**Fig 3.4.**). The second diastereoisomer was identified as (-)-*cis*-(1*S*,2*R*)-3-(*trans*-(1′*R*,2′*R*)-1′,3′-dithan-2′-yl-1′-oxy)cyclohexa-3,5-diene-1,2-diol **144** (**Scheme 4.2.**). When similar oxidations were carried out on the parent 1,3-dithiane **110** with several oxidants (DMD, $NaIO_4$ and MCPBA), the major product was always found to be the *trans*- sulfoxide. As there were only two *cis*-diol sulfoxide diastereoisomeric products from oxidation the *cis*-diol sulfide **129**, it was assumed that the second *cis*-diol sulfoxide diastereoisomer **144** had a *trans*-configuration.



129

Scheme 4.2. *Synthesis of diol sulfoxide diastereoisomers **130** and **144**.*

Unfortunately due to the instability of the dithiolane diol sulfoxide bioproducts **136** and **137**, the chemical sulfoxidation and separation of diastereoisomers was abandoned after the initial attempts. Efforts to isolate the products from this sulfoxidation *via*, cycloadduct formation, were similarly unsuccessful.

4.2. Aromatisation of chemoenzymatically synthesised *cis*-diol sulfoxides to yield phenols and catechols.

One limitation of the *cis*-dihydrodiols **122**, **125** and **140-143** proved to be their instability. They have the potential to dehydrate, the driving force of this reaction being the aromatisation of the *cis*-diol ring. Aromatisation was found to occur under conditions of high temperature and low pH. Unfortunately some sulfoxides are also prone to racemisation under acidic conditions and high temperatures. The aromatisation of *cis*-dihydrodiol sulfoxides may in principle be used in the synthesis of chiral sulfoxides containing a phenol or catechol group.

4.2.1. Phenol formation.

The crystalline diol sulfoxide **125** synthesised from the parent *cis*-diol sulfide **85** was reacted under a range of conditions, in an attempt to aromatise the diastereoisomer. Methods studied included heating solutions with p-TSA in $CHCl_3$, , NH_3 in MeOH, Et_3N in $CHCl_3$, pH 7 aqueous buffer solution in water . Samples were also absorbed on to silica gel or heated in the neat state in an effort to promote aromatisation. The progress of the reactions were monitored by TLC over an extended period of time. In each case evidence of isomerisation to a mixture of diastereoisomers **125** and **122** was observed in the presence ofphenolic products. Fortunately slight one set of reaction conditions was found to give enantiopure phenol sulfoxide **74**. Thus a small sample of **125** in 5% TFA / $CHCl_3$ solution was heated (45^{o}C for 1.5h) giving the phenolic sulfoxide **74** in a 90% yield, with only a trace of compound **84**. Sulfoxide **74** was produced with a > 98% ee by CSP HPLC analysis (Chiralcel OB column, 10% IPA/Hex, **Fig 4.3** and **Scheme 4.3.**). The CSP HPLC trace was compared with that the racemic mixture of enantiomers of **74** (α 1.13, **Appendix 2.5.**).

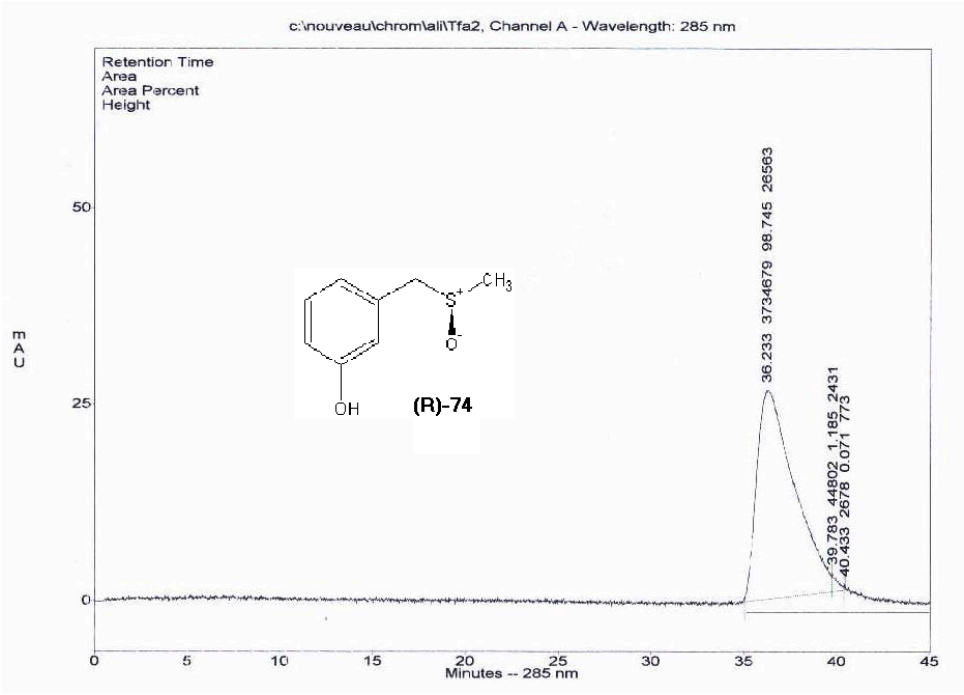
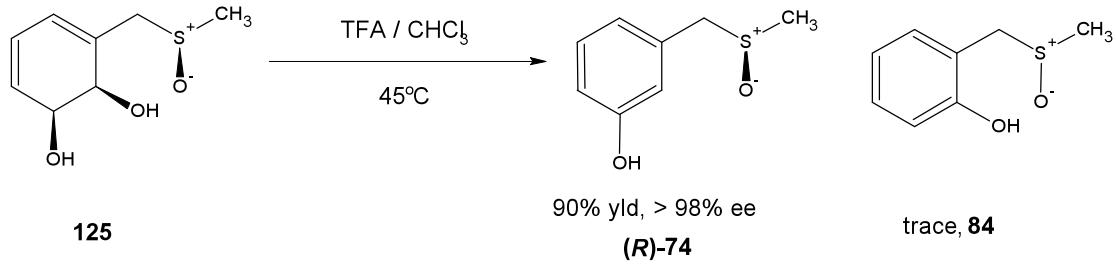


Fig 4.3. *CSP HPLC trace of enantiopure phenol sulfoxide **74**.*



Scheme 4.3. *Synthesis of enantiopure phenol sulfoxide **74**.*

Attempts were made to carry out this aromatisation reaction with crystalline diol sulfoxide **130** (*p*-TSA/$CHCl_3$). Due to the electron withdrawing nature of the sulfoxidised 1,3-dithiane ring, the aromatisation of the *cis*-dihydrodiol was relatively slow and only occurred when the solution was heated (70°C). This increased stability had earler been observed for aromatisation of othersimilar arene *cis*-dihydrodiols, containing electron withdrawing substitutents.[121] Under these conditions the rate of epimerisation appeared to be much higher than the rate of aromatisation, compared

with the relative rates observed for diol sulfoxide **125**. The reaction gave four phenolic products (^{1}H-NMR analysis), only one of which (**145**) could be fully characterised. The product was found to be racemic 2-(trans-1′-3′-dithian-2′-yl-1′-oxy)phenol **145** by X-ray crystallography (see **Fig 4.4.**). In the crystalline state the 1,3-dithiane ring adopted a conformation where the sulfoxide oxygen atom was equatorial, with no evidence of hydrogen bonding between the neighbouring hydroxyl and sulfoxide groups. The *trans*-diequatorial relationship between the phenyl and the sulfoxide groups was also evident (**Fig 4.4.**).

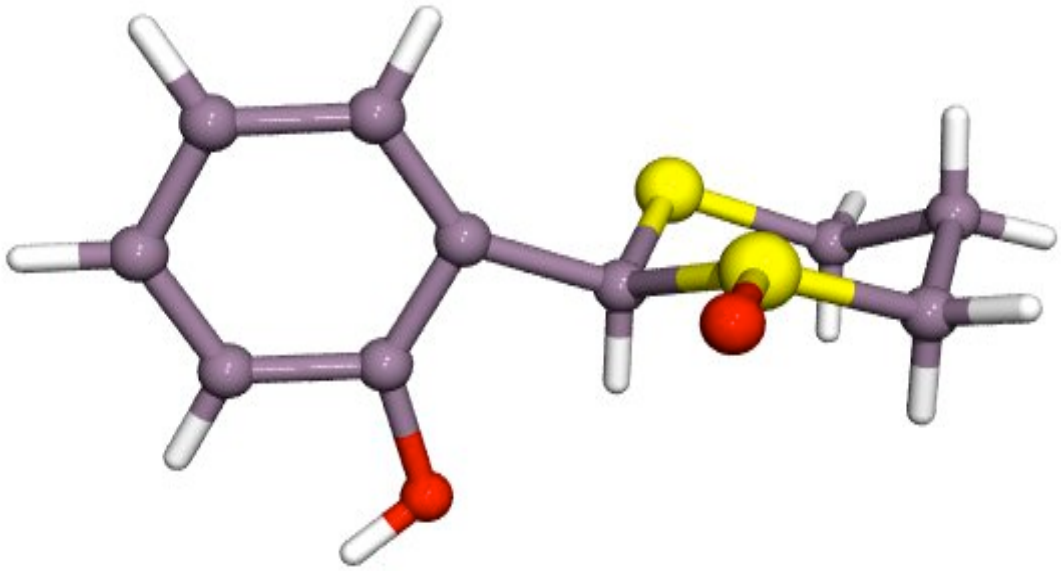
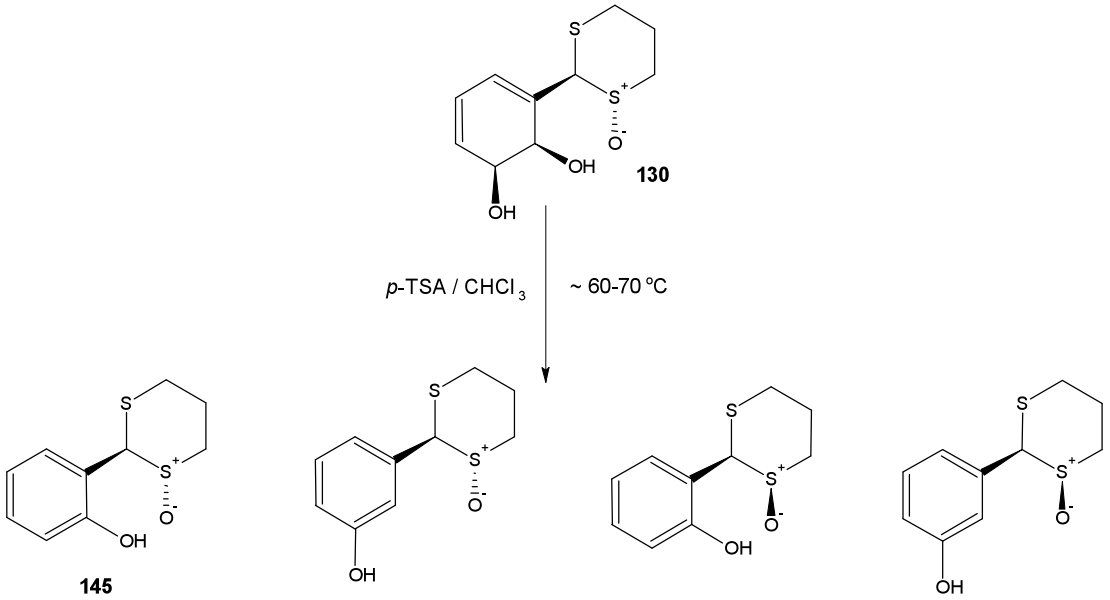


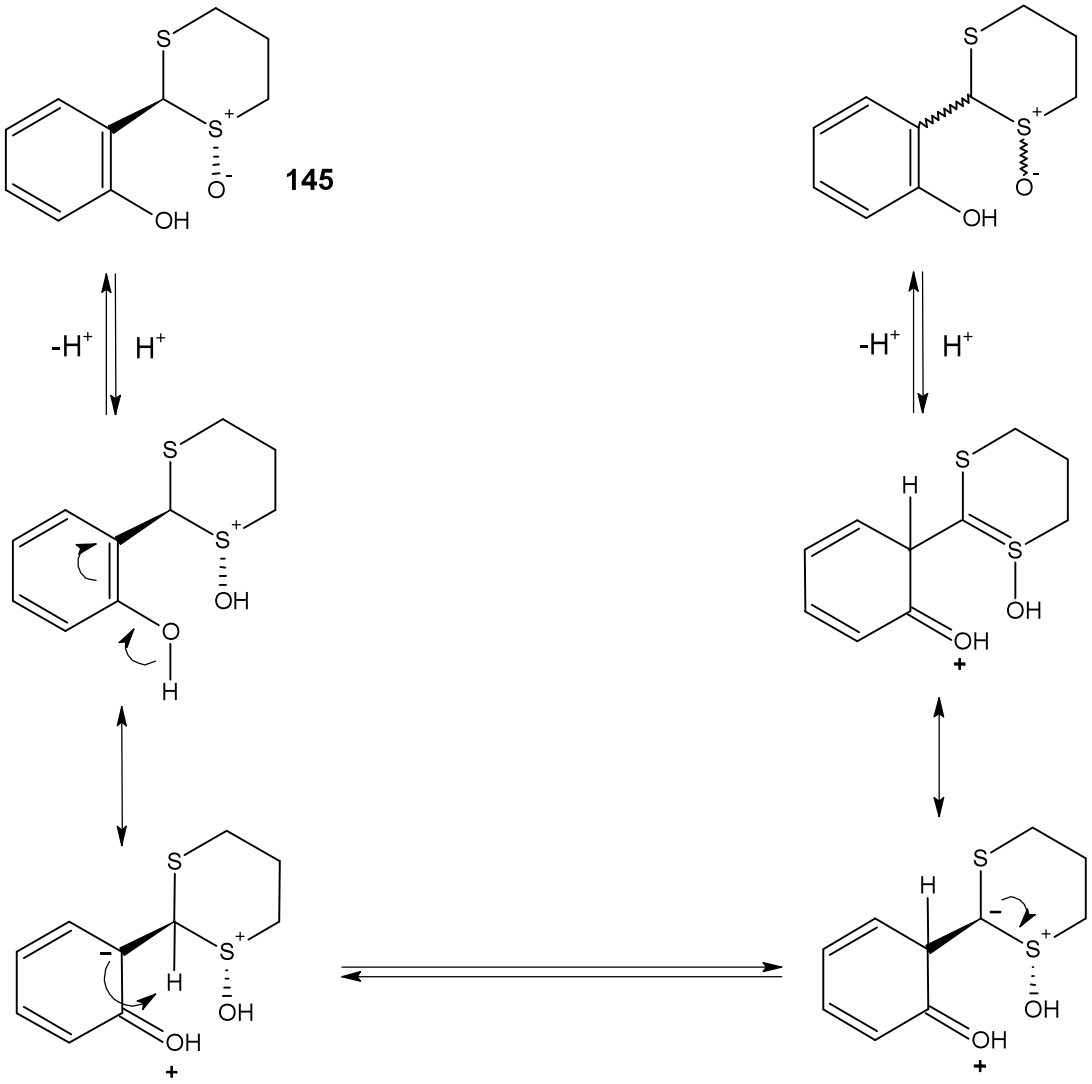
Fig 4.4. *X-ray crystal structure of (±)-2-(trans-1',3'-dithian-2'-yl-1'-oxy)phenol* ***145*** *(Dr J. F. Malone).*

NMR analysis and optical rotation measurements of the crude product mixture suggested that the other compounds were racemic *cis* / *trans* isomers of the *ortho* and *meta* phenols (**Scheme 4.4.**). The structure of the phenol sulfoxide **145** and racemisation was confirmed by X-ray crystallography.



Scheme 4.4. *Aromatisation and epimerisation of diol sulfoxide* ***130****.*

It is difficult to provide a rational mechanism to account for the racemisation of four chiral centres in the *cis*-diol sulfoxide **130**. The conversion of the *cis*-diol sulfoxide **130** to the phenolic sulfoxide **145** reduces the number of chiral centres from four to two. The possibility of protonation of the sulfoxide group to yield a sulfonium salt could facilitate epimerisation of either the phenol or *cis*-diol sulfoxides as sulfonium salts have a much lower barrier to racemisation than the parent sulfoxides. This would explain racemisation at the sulfoxide centres, although a significant contributor from a ketodiene resonance structure, in the phenol products, may result in epimerisation, essentially racemising both the remaining chiral centres (**Scheme 4.5.**). This could occur due to formation of a planar resonance structure involving a carbon-sulfur double bond between the C-2 carbon and the sulfonium sulfur atoms.



Scheme 4.5. *Total racemisation of chiral centres in phenolic sulfoxide* ***145****.*

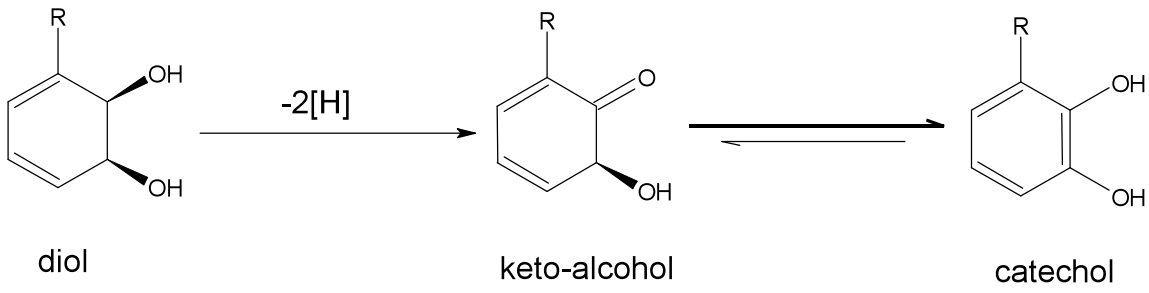
This racemisation would not occur at the same rate using a diol sulfoxide derivative **122** or **125** from benzylmethyl sulfide **81** due to the greater difficulty of formation of a carbanion intermediate on the benzylic carbon.

The diol sulfoxide diastereomer **136** obtained from the metabolism of 1,3-dithiolane **111**, showed a similar tendency to epimerise yielding multiple products when analysed by TLC and NMR.

It was observed that when the reactions were carried out at lower temperatures and with lower catalyst concentrations, epimerisation appeared to be faster than aromatisation.

4.2.2. Catechol formation.

An alternative method of aromatising the arene *cis*-dihydrodiols to yield stable chiral sulfoxides, could involve oxidation of one of the allylic hydroxyl groups to a ketone, which would tautomerise to an enol. In the case of *cis*-dihydrodiol metabolites obtained from substituted benzene substrates, the enol (catechol) was the only observable tautomer due to resonance stabilisation (**Scheme 4.6.**).

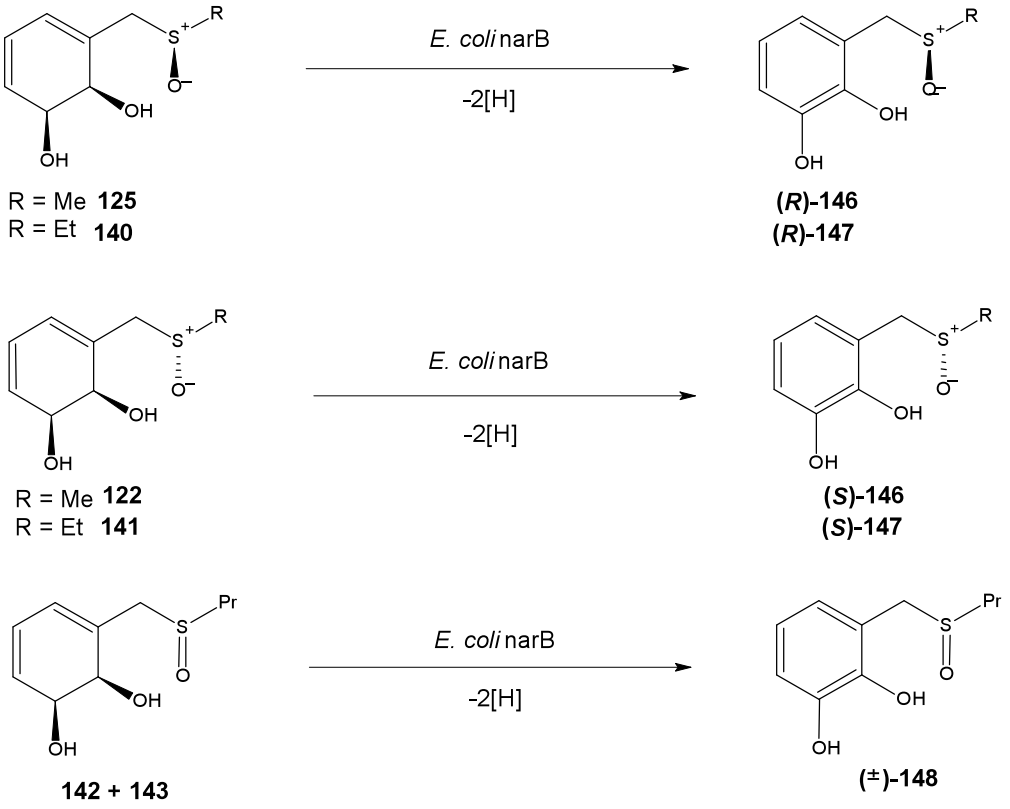


Scheme 4.6. *Oxidation of a benzene cis-dihydrodiol to a catechol.*

A reagent commonly used in dehydrogenation reactions is Pd/C, but the relatively high temperatures are required to carry out the oxidation and such conditions may be too harsh for sulfoxides and arene *cis*-dihydrodiols. Arene *cis*-dihydrodiols are more likely to dehydrate to phenols using Pd/C. Other oxidation methods such as CrO_3 or PCC may be too acidic or may require heating, and for similar reasons cannot be used. A milder enzyme-catalysed method for this reaction was examined. An *E. coli* recombinant strain[31] containing the narB gene encoding a naphthalene *cis*-dihydrodiol dehydrogenase (NDD) was available in our laboratories. This strain was tested using the synthesised diol sulfoxide diastereoisomers in order to produce enantiopure catechol sulfoxides.

The diastereoisomeric pairs of diol sulfoxides **122**, **125** and **140**, **141** and a mixture of **142** and **143** (produced from DMD oxidation of the corresponding *cis*-diol bioproducts **85**, **123** and **124**), were added to cultures of *E. coli* narB and yielded catechol sulfoxides in some cases. As the configurations of the diastereoisomeric *cis*-diol sulfoxides were known, the configurations and enantiopurity values of the derived catechol sulfoxide bioproducts were readily deduced. The diol sulfoxides **125** and **122** gave (-)-3-(*R*-methylsulfinylmethyl)benzene-1,2-diol **(*R*)-146** and (+)-3-(*S*-methylsulfinylmethyl)benzene-1,2-diol **(*S*)-146** with equal and opposite specific

optical rotations of ($[\alpha]_D$ - 70.9 ($CHCl_3$) and + 69.5 ($CHCl_3$) respectively. This experiment indicated that the *cis*-diol sulfoxide diastereoisomers were both substrates for the NDD enzyme. Similarly the diol sulfoxides **140** and **141** gave (-)-3-(*R*-ethylsulfinylmethyl)benzene-1,2-diol **(*R*)-147** and (+)-3-(*R*-ethylsulfinylmethyl)benzene-1,2-diol **(*S*)-147** with equal and opposite optical rotations ($[\alpha]_D$ - 53 and + 54, $CHCl_3$) respectively (**Scheme 4.7.**). The inseparable equal mixture of diol sulfoxide diastereoisomers **142** and **143** as expected gave 3-(propylsulfinylmethyl)benzene-1,2-diol **148** as aracemate. From these results is would appear that the NDD enzyme is unable to stereodifferentiate between diol sulfoxide diastereoisomers. The best yields of catechols were obtained for the crystalline diol sulfoxide **125** (71%). When diol sulfoxides **130** and **136** from the *P. putida* UV4 metabolism of 1,3-dithiane **110** and 1,3-dithiolane **111** were added as substrates for NDD, no catechols were produced and the substrates were recovered unchanged. This could be due to the small size of the active site of the NDD enzyme. .

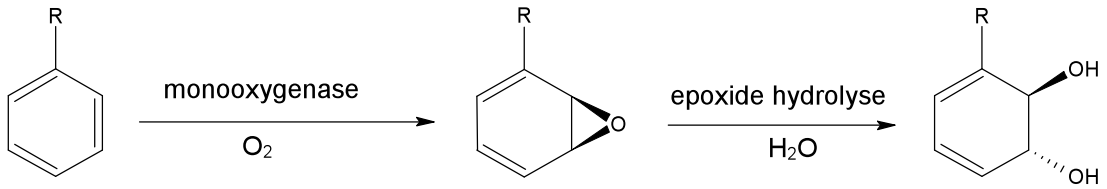


Scheme 4.7. *Catechol formation from diol sulfoxide diastereoisomer pairs **122, 125** and **140-143** using E. coli narB.*

4.3. Synthesis of *cis*-tetrahydrodiol sulfoxide diastereoisomers from the *cis*-dihydrodiol metabolite of bromobenzene.

Among the substituted benzene substrates that were studied with *P. putida* UV4 were fluoro-, chloro-, bromo- and iodo- benzenes.[122] The bioproducts were the corresponding *cis*-dihydrodiols *i.e.* *cis*-(1*S*,2*R*)-3-fluorocyclohexa-3,5-diene-1,2-diol **147**, *cis*-(1*S*,2*S*)-3-chlorocyclohexa-3,5-diene-1,2-diol **150**, *cis*-(1*S*,2*S*)-3-bromocyclohexa-3,5-diene-1,2-diol **34** and *cis*-(1*S*,2*S*)-3-iodocyclohexa-3,5-diene-1,2-diol **151**. With the exception of the fluorobenzene *cis*-dihydrodiol **149**, all other *cis*-diols were enantiopure and were obtained in excellent yields from the biotransformations. For these reasons the *cis*-dihydrodiols have proved to be very useful in chemoenzymatic synthesis (**Chapter 1**). One of the main reasons that these arene *cis*-dihydrodiols have been so useful in synthesis is the presence of the halides (*e.g.* Br- and I-) which can be interconverted into other functional groups. This halide substitution procedure increases the number and types of enantiopure *cis*-dihydrodiols that can be synthesised and subsequently used as chiral synthons.

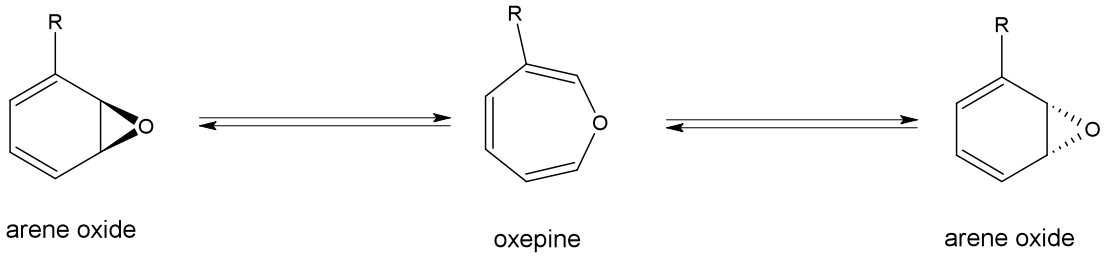
Monooxygenases have earlier been shown to catalyse the oxidation of arenes into arene oxides that are in turn converted to *trans*-dihydrodiols *via* hydrolysis using epoxide hydrolase enzymes (**Scheme 4.8.**).



Scheme 4.8. *Conversion of monocyclic arenes to arene oxides and trans-dihydrodiols.*

These arene oxides and *trans*-dihydrodiols have been implicated as the initial metabolites formed during the metabolism of carcinogenic PAHs. For this reason there has been much interest in studying their synthesis and their physical and chemical properties. During the latter studies it was found that arene oxides of monosubstituted benzenes have a low barrier to racemisation.[7] This is due to the arene oxide being spontaneously isomerised to the corresponding oxepine *via* an

electrocyclic rearrangment involving cleavage of the epoxide C-C bond (**Scheme 4.9.**).

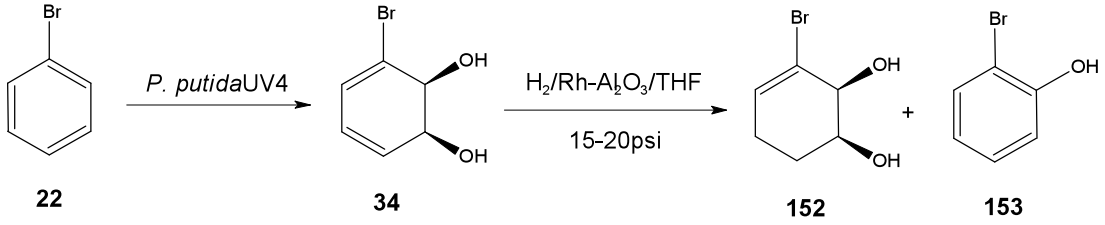


Scheme 4.9. *Spontaneous racemisation of substituted benzene oxide enantiomers via oxepine valence tautomers*.

Earlier work carried out in these laboratories[7] has involved the synthesis and racemisation of both arene oxides and arene dioxides. It has also been shown that arene *trans*-dioxides can be converted into *trans*-dihydrodiols.

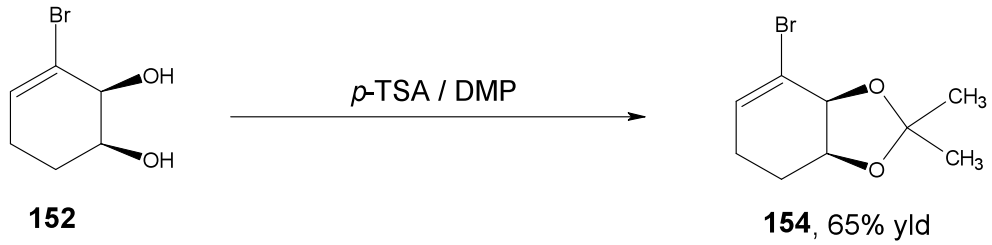
In this study, the synthesis of possible synthetic precursors to arene oxides and *trans*-dihydrodiols containing exocyclic sulfoxide centres has been attempted.

The first factor was the choice of starting material. The *cis*-dihydrodiol metabolite **34** obtained from the metabolism of bromobenzene **22** with *P. putida* UV4 was selected as it was relatively stable and the bromine atom was readily replaceable. It was also available in higher yields compared with less the stable iodo-substututed bioproduct **151**. In a preliminary experiment, *cis*-diol **34** was partially hydrogenated using H_2/Rh-Al_2O_3 in THF at 15-20 psi and two products were isolated. *cis*-(1*S*,2*S*)-3-Bromocyclohex-3-ene-1,2-diol **152** was obtained in a 40% yield and 2-bromophenol **153** in a 35% yield (see **Scheme 4.10.**). Clearly the presence of the phenolic product was due to dehydration of the *cis*-dihydrodiol under the reaction conditions. When this reaction was repeated on a larger scale, but using a more dilute solution, the yields of the tetrahydrodiol **152** were found to be higher (~ 80% yield), and phenol **153** was obtained as a very minor product.



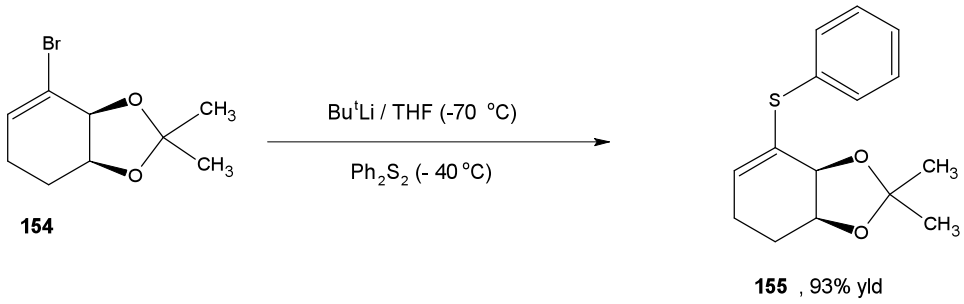
Scheme 4.10. *Partial hydrogenation of cis-dihydrodiol* ***34***.

Protection of the *cis*-dihydrodiol **152** as an acetonide derivative was found to facilitate substitution of the bromine with a thiophenoxide group (**Scheme 4.11.**).



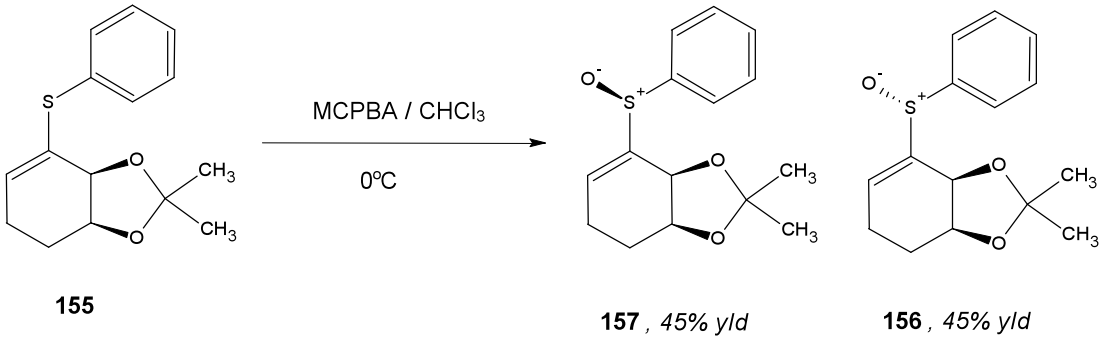
Scheme 4.11. *Protection of cis-tetrahydrodiol* ***152*** *by formation of an acetonide*.

The *cis*-diol **152** was converted to the acetonide *cis*- (3a*S*,7a*S*)-7-bromo-2,2-dimethyl-3a,4,5,7a-tetrahydro-1,3-benzodioxole **154** (65% yield) by reacting with dimethoxy propoane (DMP) in the presence of an acid catalyst. This acetonide **154** was in turn reacted with Bu^tLi in THF at – 70°C followed by the addition of Ph_2S_2 and allowed to warm to – 40°C giving the thioether product **155** (93% yield, **Scheme 4.12.**). Isolation and purification of the thioether **155** by PLC was aided by the presence of the benzene chromophore.



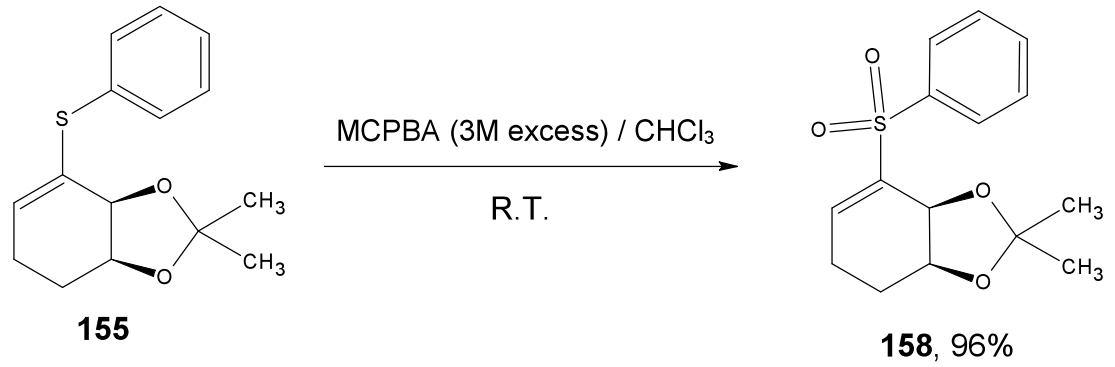
Scheme 4.12. *Substitution of the vinylic bromine with a sulfanyl group*.

In the next step the sulfur atom was oxidised using either DMD or MCPBA. The DMD oxidation was monitored by TLC and gave only sulfoxide products. These sulfoxides were separated by PLC. On the basis of NMR and MS data the sulfoxides were identified as the *cis*-tetrahydrodiol sulfoxides *cis*-(3a*S*,7a*S*)-2,2-dimethyl-7-(*R*-phenylsulfinyl)-3a,4,5,7a-tetrahydro-1,3-benzodioxole **156** and *cis*-(3a*S*,7a*S*)-2,2-dimethyl-7-(*S*-phenylsulfinyl)-3a,4,5,7a-tetrahydro-1,3-benzodioxole **157** (**Scheme 4.13.**).



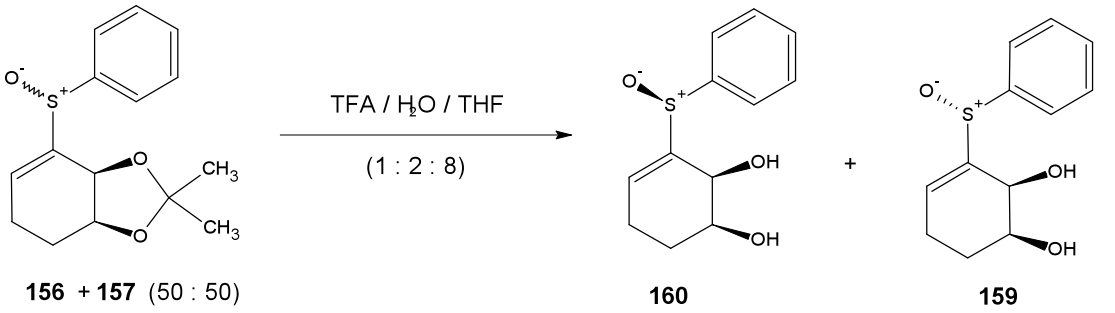
Scheme 4.13*. MCPBA oxidation of a sulfide* ***155*** *and separation of the sulfoxide diastereoisomers* ***156*** *and* ***157****.*

The MCPBA oxidation was carried out in $CHCl_3$ at room temperature and yielded three products, the first two products being spectrally identical to the sulfoxide diastereoisomers **156** and **157**. The third very minor product appeared to be the sulfone *cis*-(3a*S*,7a*S*)-2,2-dimethyl-7-(phenylsulfonyl)-3a,4,5,7a-tetrahydro-1,3-benzodioxole **158** (**Scheme 4.14.**). This was confirmed by addition of a large excess of oxidant that gave one product, sulfone **158** (96% yield). No evidence of an epoxide product was observed. Over-oxidation with DMD also yielded a spectrally similar compound, without epoxide formation. This observation is consistent with the electron withdrawing nature of the sulfone group which prevented epoxidation.



Scheme 4.14. *Oxidation of sulfide* ***155*** *to sulfone* ***158***.

The final step in the synthesis involved removal of the acetonide protecting group from the diastereoisomeric mixture. This was achieved by heating a mixture of the sulfoxide diastereoisomers **156** and **157** at 40°C in a solution of 1 :2 :8 TFA/H_2O/THF and separation by PLC giving the diol sulfoxide products (-)-*cis*-(1*S*,2*S*)-3-(*R*-phenylsulfinyl)cyclohex-3-ene-1,2-diol **159** and (-)-*cis*-(1*S*,2*S*)-3-(*S*-phenylsulfinyl)cyclohex-3-ene-1,2-diol **160** in 70% overall yield. A specific optical rotation values ($[\alpha]_D$) of – 7 ($CHCl_3$) and – 89 ($CHCl_3$) were found for the less polar crystalline metabolite for the more polar metabolites respectively (**Scheme 4.15.**).



Scheme 4.15. *Deprotection of sulfoxide and separation of the resulting diastereoisomers* ***159*** *and* ***160***.

The absolute configurations of the *cis*-tetrahydrodiol sulfoxide product **160** was determined by X-ray crystal structure analysis, using the anomalous dispersion

method, on the less polar crystalline product (**Fig 4.5.**). In the crystalline state diastereoisomer **160** adopted a conformation where the sulfoxide group was equatorial, with the hydroxyl group on C-1 in a pseudo-equatorial position and C-2 – hydroxyl group in a pseudo-axial position. There is also some evidence of hydrogen-bonding between hydroxyl at C-2 and and the sulfoxide oxygen atom. The absolute configuration at the sulfoxide centre was found to be (*S*). This identified the product (-)-*cis*-(1*S*,2*S*)-3-(*S*-phenylsulfinyl)cyclohex-3-ene-1,2-diol **160**. The configuration of the other diastereoisomer was thus deduced to be (-)-*cis*-(1*S*,2*S*)-3-(*R*-phenylsulfinyl)cyclohex-3-ene-1,2-diol **159**.

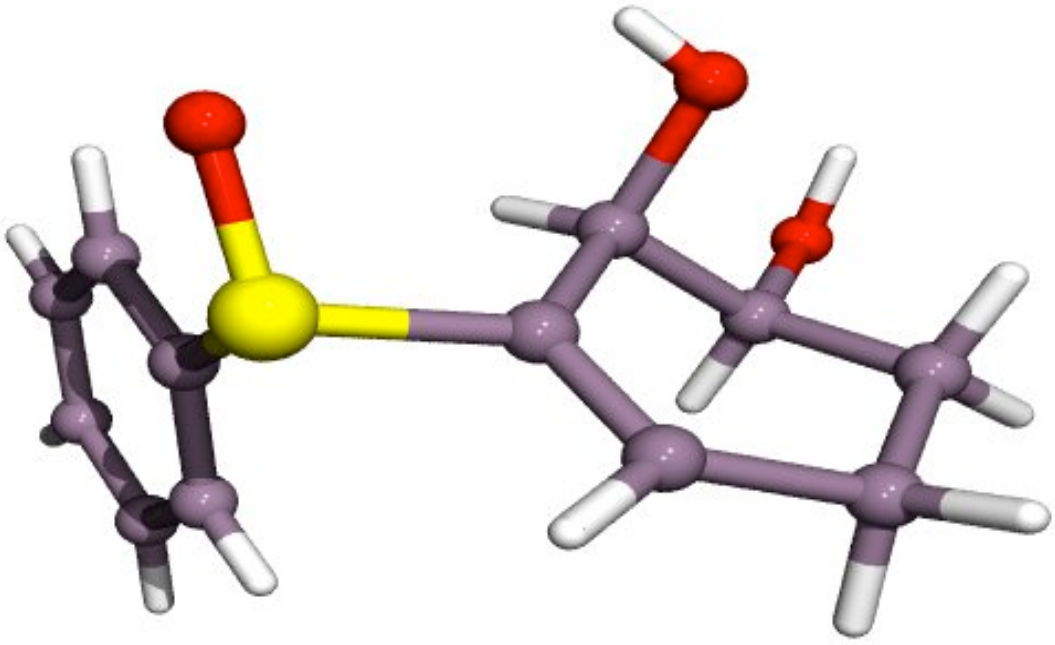
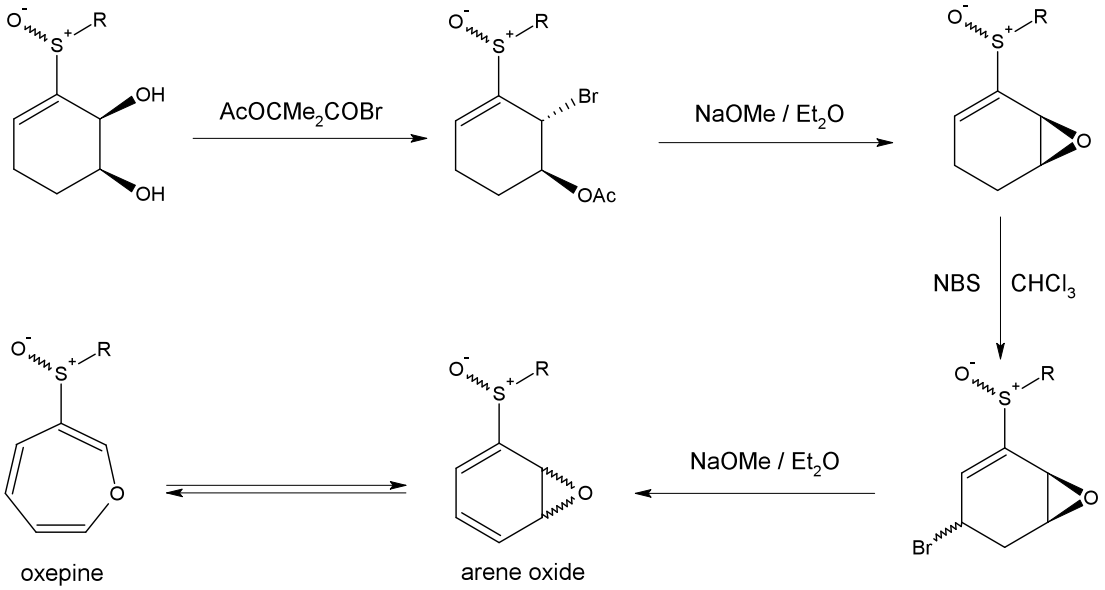


Fig 4.5. *X-ray crystal structure of (-)-cis-(1S,2S)-3-((S)-phenylsulfinyl)cyclohex-3-ene-1,2-diol* ***160*** *(Dr J. F. Malone).*

The dihydroxylated sulfoxides **159** and **160** provide the first examples of chemoenzymatically synthesised *cis*-tetrahydrodiol sulfoxides, derived from the dioxygenase-mediated metabolism of monosubstuted arenes. As *cis*-tetrahydrodiol sulfoxides these compounds **159** and **160** were found to be much more stable than the corresponding *cis*-dihydrodiols. They also provide a new type of hydroxy sulfoxide with potential as chiral ligands for asymmetric asymmetric alkylation reactions using diethylzinc. The *cis*-tetrahydrodiol could also as synthetic precursors of arene oxides and *trans*-dihydrodiols each containing chiral sulfoxide groups. The potential relevance of these intermediates in synthesis will discussed briefly.

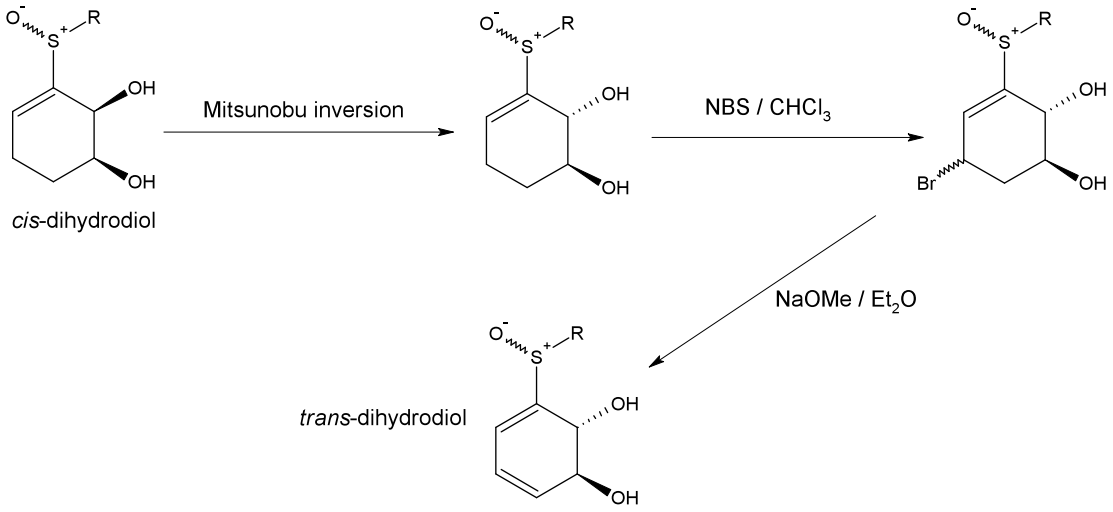
As discussed in Chapter 2, diethylzinc reactions with aldehydes may be catalysed by chiral alcohol sulfoxides. Previously it has been possible to substitute one of the less hindered hydroxyl groups on *cis*-dihydrodiols and *cis*-tetrahydrodiols with methoxyl groups or silyl ether groups. The X-ray crystal structure of the *cis*-diol sulfoxide **160** also shows possible hydrogen bonding between the hydroxyl group on C-2 and the sulfoxide. It should be simple to selectively protect the -OH group on C-1 leaving the C-2 -OH group free to form intramolecular hydrogen-bonds, or to form a chiral complex with diethylzinc.

It should also be possible to synthesise and study the physical and chemical properties of arene oxides derived from diastereoisomers **159** and **160**. In previous studies on the synthesis of arene oxides and their racemisation through oxepine formation the synthetic route involved formation of a tetrahydrodiol similar to compound **159** and **160**. This approach may also be applied to the synthesis of arene oxides containing sulfoxide groups (**Scheme 4.16.**). This synthetic scheme could involve formation of a bromoacetate by reacting the diol with $AcOCMe_2COBr$, followed by conversion to the epoxide using NaOMe. The epoxide could be brominated with NBS, and HBr removed with NaOMe giving an arene oxide. This technique has been successfully applied to other tetrahydrodiols in these laboratories. The inclusion of the fixed sulfoxide chiral centre in this scheme could aide the study of the epimerisation of the arene oxide by techniques such as ^{1}H-NMR spectroscopy as the diastereoisomers would have different chemical and physical properties.



Scheme 4.16. *Application of a cis-tetrahydrodiol sulfoxide diastereoisomer in the synthesis of epimerising arene oxide/oxepine sulfoxide diastereoisomers.*

From the diastereoisomers **159** and **160** it should also be possible to synthesise the corresponding *trans*-dihydrodiol sulfoxides. One of the well-known methods in synthetic organic chemistry of inverting the stereochemistry of an alcohol is the 'Mitsunobo inversion'. Theoretically, using this method, it is possible to synthesise arene *trans*-dihydrodiols from arene *cis*-dihydrodiols by inverting the stereochemistry of the 2-hydroxyl. It has been demonstrated by Hudlicky *et al*[123] that the direct inversion of the C-2 chiral centre bearing a hydroxyl group in *cis*-dihydrodiols is not possible due to the unstable nature of arene *cis*-dihydrodiols. It is however possible to synthesise *trans*-dihydrodiols *via* a Mitsunobo inversion on the *cis*-tetrahydrodiol, followed by bromination and dehydrobromination to give the arene *cis*-dihydrodiol.[7] This technique may be applied to diol sulfoxides similar to **159** and **160** (**Scheme 4.17.**). This could allow the study of the physical and chemical properties of the potential carcinogenic *trans*-dihydrodiol metabolites derived from organosulfur compounds.



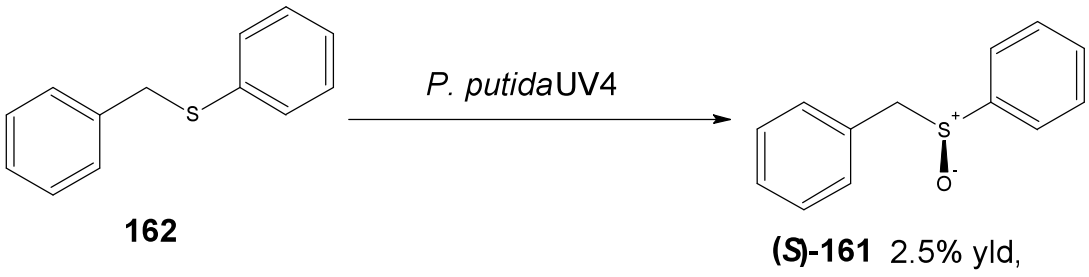
Scheme 4.17. *Synthesis of trans-dihydrodiols from a cis-tetrahydrodiols*.

4.4. Synthesis of potential hydroxy sulfoxide ligands from an optically pure benzyl sulfoxide derived from the 'Andersen method'.

As briefly discussed in Chapter 1, chemoenzymatically synthesised enantiopure sulfoxide **(*S*)-77** was used in the enantioselective addition of diethylzinc to benzaldehyde **101**. The results seemed promising even though reaction conditions had not yet been optimised. In previous sections, a range of hydroxylated sulfoxides have been synthesised chemoenzymatically, including studies into the dioxygenase-mediated metabolism of dialkyl sulfides, containing benzylic carbon atoms, in an attempt to synthesise enantioenriched dialkyl sulfoxides using an enzyme-catalysed asymmetric synthesis. In this section it will be demonstrated how functionalised sulfoxides, with potential as chiral ligands, can be synthesised from an optically pure benzyl sulfoxide, benzyl-*p*-tolyl sulfoxide **91**.

This sulfoxide **91** was chosen as it was a benzylic sulfoxide and this sulfoxide type may be functionalised at the benzylic carbon atom, due to the ease of formation of stabilised carbanions. The inclusion of the second aromatic ring avoided the possibility of any side reactions with stabilised alkyl groups as the second sulfoxide substituent.

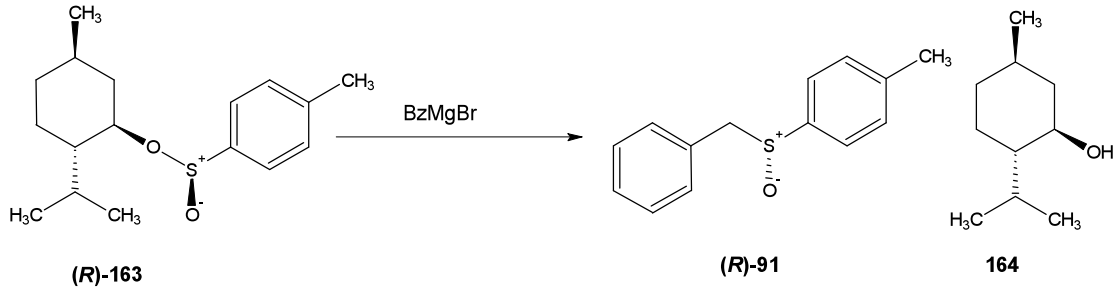
Benzylphenyl sulfoxide **161**, a sulfoxide similar in structure to sulfoxide **91** has been produced in an enantioenriched form by the dioxygenase-mediated metabolism of the parent sulfide **162** using *P. putida* UV4 (see **Scheme 4.18.**).[99]



Scheme 4.18. *Dioxygenase-mediated metabolism of sulfide* ***162*** *using P. putida UV4.*

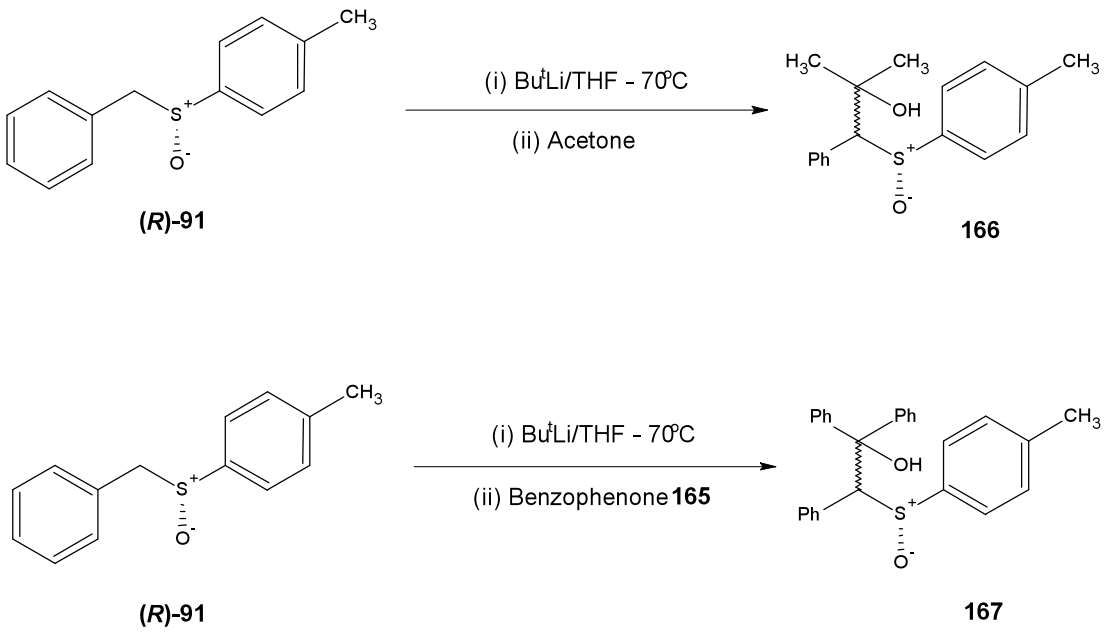
Although the sulfoxide **(*S*)-161** was produced with a high ee value, the isolated yield is very poor.

It was also hoped that the sulfoxide **91** might be a good substrate for the DMSO reductase-containing strains of bacteria which would provide enough of an enantiopure sample for further synthetic studies. Unfortunately no resolution was observed, therefore an optically pure sample of **(*R*)-91** was provided from a previous synthesis utilising the 'Andersen method'. This involved the reaction of commercially available (*R*)-menthyl-*p*-tolyl sulfinate **(*R*)-163** with benzyl magnesium bromide (Grignard reagent, **Scheme 4.19.**) yielding sulfoxide **(*R*)-91**.



Scheme 4.19. *Synthesis of enantiopure sulfoxide* ***(R)-91*** *using the 'Andersen method'.*

The enantiopure sulfoxide **(*R*)-91** was then reacted with mixtures of Bu^tLi/THF followed by addition acetone or benzophenone **165** at – 70°C (**Scheme 4.20.**).



Scheme 4.20. *Functionalisation of sulfoxide* ***(R)-91***.

The products from these reactions were both produced as single diastereoisomers, the absolute configurations of these were determined by X-ray crystallography, using the anomalous dispersion method (**Figs 4.6.** and **4.7.**). The X-ray crystal structure of crystalline **166** ($[\alpha]_D$ + 372, $CHCl_3$) showed the aromatic substituents facing each other, with no evidence of hydrogen-bonding, due to steric interaction. The X-ray analysis confirmed the structure absolute configuration of sulfoxide **166** to be (+)-(1*S*)-2-methyl-1-(*R*-4-methylphenylsulfinyl)-1-phenylpropan-2-ol.

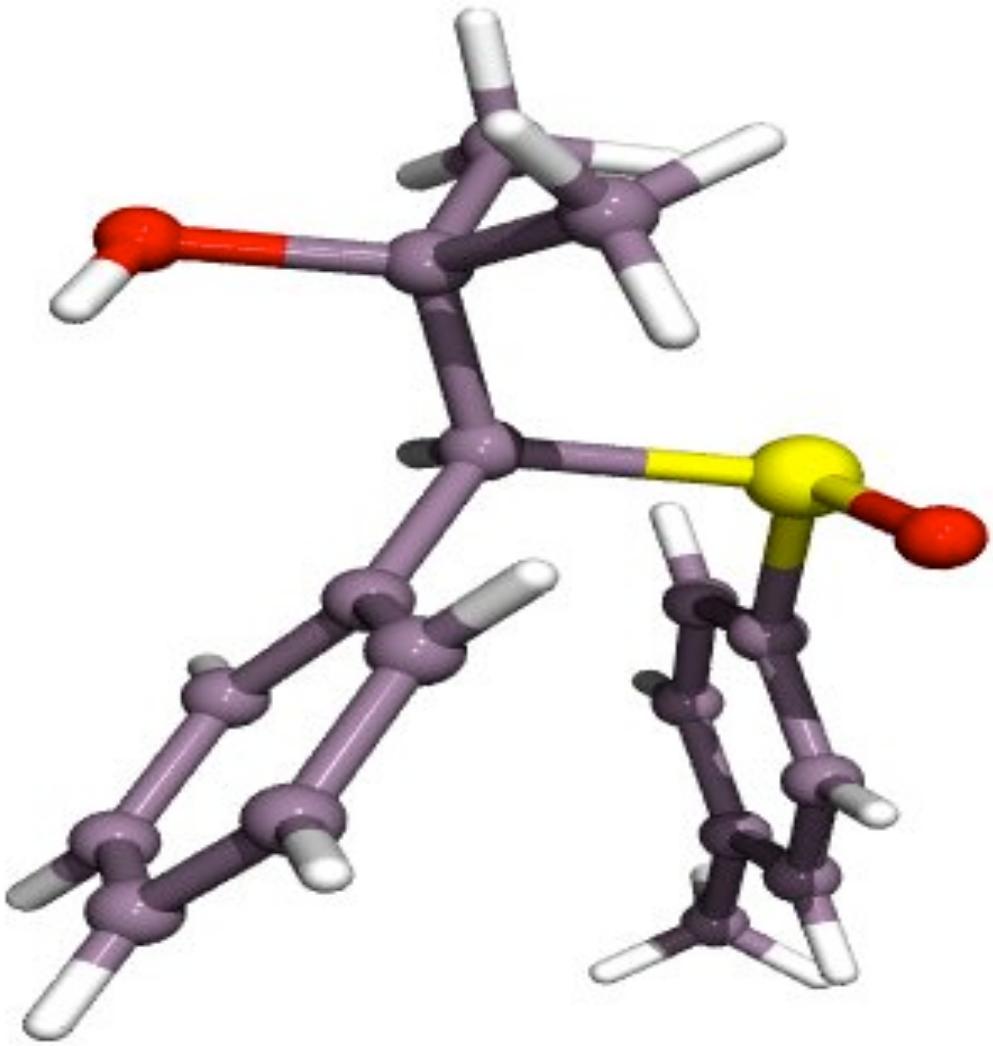


Fig 4.6. *X-ray crystal structure of (+)-(1S)-2-methyl-1-(R-4-methylphenylsulfinyl)-1-phenylpropan-2-ol* ***166*** *(Dr J. F. Malone).*

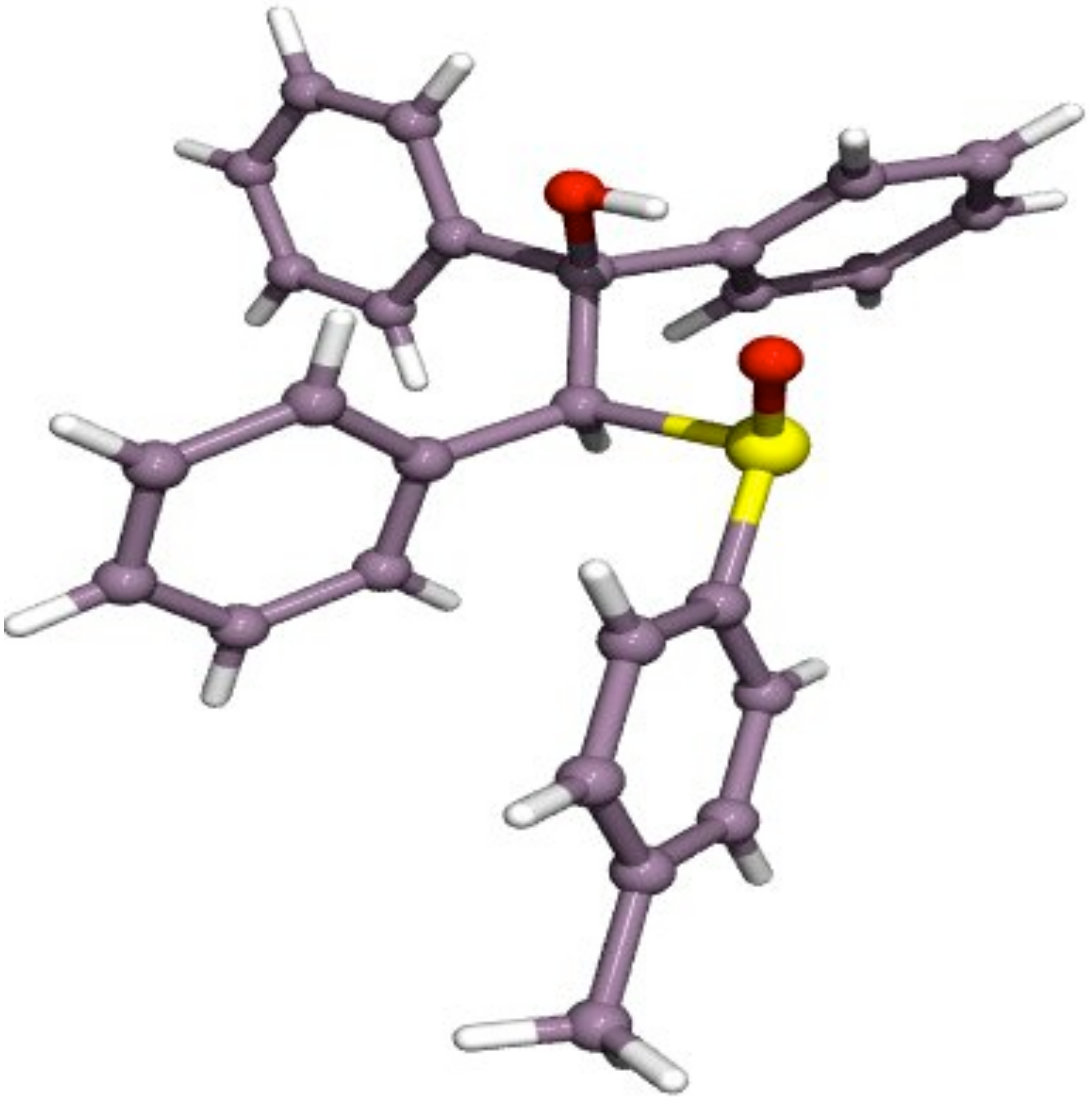
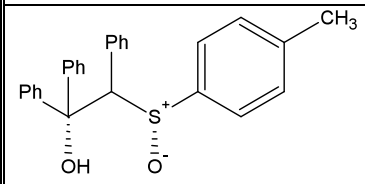
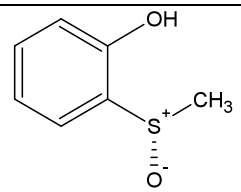
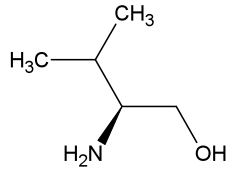
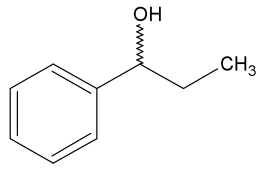


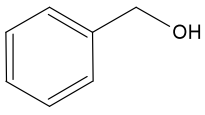
Fig 4.7. *X-ray crystal structure of (+)-(2S)-2-(R-4-methylphenylsulfinyl)-1,1,2-triphenylethan-1-ol* ***167*** *(Dr J. F. Malone).*

The X-ray structure of compound **167** ($[\alpha]_D$ + 137, $CHCl_3$) showed the aromatic substituents *cis* to each other, with evidence of hydrogen-bonding between the hydroxyl group and the sulfoxide oxygen atom. The X-ray analysis confirmed the structure and configuration of sulfoxide **167** to be (+)-(2*S*)-2-(*R*-4-methylphenylsulfinyl)-1,1,2-triphenylethan-1-ol.

Hydroxy sulfoxide **167** was then tested under the same conditions as for sulfoxide **(*S*)-77**, (**Chapter 2**) for applicability as a ligand in asymmetric Et_2Zn reactions. The results are presented in **Table 4.1.**

Ligand	Ee (%) of 105	Isolated Yield (%)			
		107	108	104	109
 **167**	16.3	56	15	15	7
 **(*S*)-77**	27.0	30	21	17	10
 **(*S*)-106**	8.7	79	8	0.5	_


107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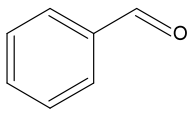

104

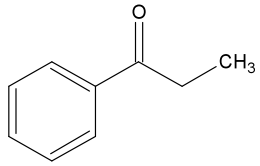

109

Table 4.1. *Enantioselective addition of Et_2Zn to benzaldehyde **104**.*

The results in **Table 4.1.** showed that the new hydroxy sulfoxide **167** has the ability to catalyse the enantioselective addition of Et_2Zn to benzaldehyde **101**. Although the ee values are lower, the yields of the byproducts **108** and **109** have decreased in comparison to the overall yield. The ee value is however still higher that that obtained by the standard ligand **(*S*)-106**, which in the literature gives a higher value than in this preliminary study.

4.5. Conclusion.

In this and previous chapters it has been shown that a wide range of enantioenriched functionalised sulfoxides, such as hydroxylated sulfoxides, can be synthesised chemoenzymatically, using combinations of DMSO reductase dioxygenase and *cis*-diol dehydrogenase enzymes allied to chemical syntheses. Some of these sulfoxides may have been produced as metabolites, whose structures and absolute configurations can tell us more about the chemo-, regio- and enantioselectivities of attack of the enzymes under study. Other sulfoxides produced have been chemoenzymatically synthesised from these metabolites. Other uses for these sulfoxides may be as chiral auxiliaries or ligands in reactions such as the asymmetric diethyl zinc reactions. Two such sulfoxides **77** and **167** have already shown potential for this type of reaction.

Chapter 5. Experimental.

5.1. General experimental details.

Equipment used in the characterisation of products is summarised in **Table 5.3.**, along with explanatory comments.

Chromatographic techniques employed in purification are listed below (**Table 5.1.**). Visualisation of bands/spots was at 254nm with a Spectroline ENF-260C/F UV lamp.

Method	Stationary phase
Analytical TLC	Merck Kieselgel $60F_{254}$ plates
Preparative TLC (PLC)	Glass plates (20cm x 20cm) coated with Merck Kieselgel Pf_{254} (21g in 59ml water)
Flash Chromatography	Merck Kieselgel 60 (230-400 mesh) at a flow rate of c.a. 2.5cm/minute

Table 5.1. *Silica based chromatographic techniques used in analysis and purification*

Solvents for chromatography and recrystallisation were as specified for each compound and were distilled before use. When anhydrous solvents were used, the following procedures were followed (**Table 5.2.**).

Acetone	Distilled from potassium permanganate and stored over potassium carbonate.
Benzene	Stored over sodium wire.
Dichloromethane	Distilled from phosphorous pentoxide and stored over 4A molecular sieves.
DMF	Distilled from phosphorous pentoxide and stored over 4A molecular sieves.
Ether	Stored over sodium wire.
Pyridine	Stored over potassium hydroxide pellets.
THF	Distillation over sodium/benzophenone.

Table 5.2. *Drying procedures for solvents*.

Analysis	Instrument / comment
Melting points (mp)	Reichert block; uncorrected
^{1}H-NMR-300 & 500MHz	General Electric QE 300 or GN 500, Bruker Avance DPX-300 or DRX-500; All coupling constants (*J*) are measured in Hertz; All chemical shifts are reported in ppm from TMS.
^{13}C-NMR-125MHz	Bruker Avance DRX-500.
Mass spectra	Recorded at 70eV on an AE1-MS902 instrument updated by V. G. Autospeck instruments, using a heated inlet system.
Accurate molecular weights	Recorded on an AE1-MS902 instrument updated by V. G. Autospeck instruments by the peak matching method using perfluorokerosene as standard and were accurate to within ± 0.000006 a.m.u.
Elemental Analyses	Perkin-Elmer 2400 CHN microanalyser and compounds quoted gave elemental analysis within ± 0.5% of the theoretical values.
$[\alpha]_D$	Perkin-Elmer automatic precision polarimeter Model 241; Solvent and concentration (g/100cm^3) specified for each compound. Recorded at the sodium D line (598nm) and at ambient temperature.
Circular Dichroism (CD) spectra	Jasco J-720 instrument; methanol solvent (spectroscopic grade). Sample concentration (ca. 0.5mg/cm^3), $\Delta\varepsilon$ in cm^{2}mmol^{-1}.
HPLC	Beckman System gold® 128 solvent module and 168 detector, Pye Unicam LC-XPD pump and LC-UV detector, Shimadzu LC-6A connected to a Hewlett Packard Diode Array detector.
X-ray crystallogrophy	Siemens P3/V2000 diffractometer.

Table 5.3.*Equipment used in characterisation*.

5.2. General biotransformation procedure.

A typical shake flask biotransforamtion procedure for *P. putida* UV4 is detailed below.

P. putida UV4 was streaked aseptically on to L-agar plates and incubated for 15hrs at 30^{o}C. Single colonies from the plates were streaked on two minimal salts (MS) plates (with paper grids attached and one of them also containing indole) using sterile tooth picks. After 24hrs incubation, the indole plate was examined. The relative intensities of the indigo spots on the plate indicated the most dioxygenase-active colonies and were selected for carrying out the biotransformation.

Dioxygenase-active colonies (indole-test positive) were inoculated into 20 Erlenmeyer flasks (250cm^3) containing minimal growth medium (50cm^3) and 12.5% w/w sodium pyruvate solution (2cm^3) followed by incubation on a rotary shaker (250 rev/min) at 30 °C for 7hrs. The turbid solutions were transferred into 2L Erlenmeyer

flasks containing minimal growth medium (375cm^3) and 12.5% w/w/ sodium gluconate solution (15cm^3) and were incubated on a rotary shaker at 30^{o}C overnight. Centrifugation of the culture suspensions at 7000 rpm for 15min removed the bacterial cells and yielded the aqueous supernatant. This aqueous mixture of bioproducts was concentrated under reduced pressure at ~ 35 ^{o}C and the residue repeatedly extracted with ethyl acetate. The extract was dried (Na_2SO_4) and solvent removed under reduced pressure to yield the crude biotransformation products for purification.

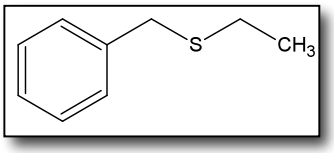
Large scale biotransformations were performed using Electrolab 7- and 10L- and LH/Inceltech 20- and 50L-fermenters (**Table 5.4.**).

Make	**Volume (working volume)**	**Sterilisation method**	**Heating method**	**Description**
Electrolab	7L (5L)	Autoclave	None (used at room temperature)	Glass vessel with a two step rushden turbine, sample port and punctured bar oxygen inlet.
Electrolab	10L (7-8L)	Autoclave	None (used at room temperature)	As above but was also fitted with pH monitor, oxygen tension and temp. probes.
LH/Inceltech	20L (18L)	Self-sterilizing	External electrical heating element.	As for Electrolab (10L).
LH/Inceltech	50L (33L)	Self-sterilizing	Steam operated heating system.	As for Electrolab (10L).

Table 5.4. *Details of fermenters*.

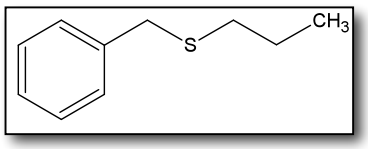
For 10L and smaller biotransformations, batch centrifugation at 7000 rpm for 15mins was used to remove the cells post biotransformation. For larger volumes, continuous centrifugation was employed using a Sharples steam operated continuous centrifuge. This consisted of a tube spinning at 45000 rpm through which the cell suspension was passed. The bacteria are retained in the spinning tube and the cell free supernatant collects at the end. Aqueous extracts were generally stored awaiting extraction at 4^{o}C for a few weeks, depending on the stability of the bioproducts.

5.3. Synthesis of substrates for metabolism with *P. putida* UV4.



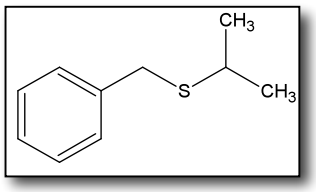
Benzylethyl sulfide 87.

Benzyl mercaptan (20g, 0.161mol) was added to a stirred solution of sodium (4.2g, 0.175mol) dissolved in anhydrous ethanol (150cm^3). The reaction mixture was stirred for a further 20 min under an atmosphere of nitrogen gas. Ethyl iodide (27g, 0.173mol) was added and the reaction mixture refluxed (6hr) under anhydrous conditions. The solvent was evaporated off, the residue extracted with ether and washed with water. The extract was dried (Na_2SO_4), the solvent removed under reduced pressure and the residue was distilled to give sulfide **87** (23.5g, 96%) as a colourless oil; bp 155-157 ^{o}C (lit.[124] bp 35-36 ^{o}C /15 mmHg); δ_H (500 MHz, $CDCl_3$) 1.23 (3 H, t, *J* 7.4, Me), 2.43 (2 H, q, *J* 7.4, C*H*$_2$Me), 3.72 (2 H, s, ArC*H*$_2$), 7.22-7.25 (1 H, m, Ar), 7.28–7.31 (4 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 14.27, 25.14, 35.81, 126.77, 128.35, 128.71, 138.52; m/z (ES) 191 ([M+39]$^+$, 100%).



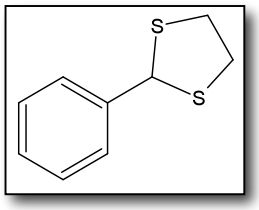
Benzylpropyl sulfide 88.

Benzyl mercaptan (20g, 0.161 mol) was added to a stirred solution of sodium (4.2g, 0.175 mol) dissolved in dry ethanol (150 cm^3). Propyl iodide (29.4g, 0.173mmol) was then added to the stirring reaction mixture. After refluxing the reaction mixture (2hr) under anhydrous conditions the solvent was evaporated off, the residue extracted with ether and washed with water. The extract was dried (Na_2SO_4) and the solvent removed under reduced pressure. The crude product was distilled to yield sulfide **88** (24.5g, 92%) as a colourless oil; bp 165 ^{o}C (lit.[125] bp 112 ^{o}C /15 mmHg); δ_H (500 MHz, $CDCl_3$) 0.95 (3 H, t, *J* 7.3, Me), 1.57 (2 H, m, C*H*$_2$Me), 2.39 (2 H, t, *J* 7.3, C*H*$_2$Et), 3.70 (2 H, s, ArC*H*$_2$), 7.22–7.25 (1 H, m, Ar), 7.28–7.31 (4 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 16.21, 25.27, 36.15, 38.96, 129.57, 131.15, 131.54, 141.41; m/z (ES) 205 ([M+39]$^+$, 100%).



Benzyl *i*-propyl sulfide 89.

Benzyl mercaptan (4g, 16mmol) was added to a stirred solution of sodium (0.84g, 18mmol) dissolved in dry ethanol (50cm^3). Isopropyl iodide (3g, 17mmol) was then added to the stirring reaction mixture. After refluxing the reaction mixture (2hr) under anhydrous conditions the solvent was evaporated off, the residue extracted with ether and washed with water. The extract was dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue was purified by column chromatography (hexane) yielding sulfide **89** (4.8g, 90%) as a colourless oil; bp 65^{o}C /2mmHg (lit.[126] 95-98^{o}C 10mmHg); R_f 0.85 (Hexane); δ_H (500 MHz, $CDCl_3$) 1.25 (6 H, d, *J* 6.7, Me), 2.80 (1 H, sept, *J* 6.7, CH), 3.74 (2 H, s, CH_2), 7.22–7.25 (1 H, m, Ar), 7.28–7.34 (4 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 22.41, 33.55, 34.45, 126.08, 127.73, 128.04, 138.06; m/z (ES) 205 ([M+39]$^+$, 100%).



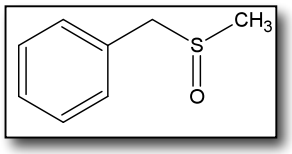
2-Phenyl-1,3-dithiolane 111.

A solution of BF_3-Et_2O complex (6cm^3, 0.05mmol) was added, dropwise with stirring, to a solution of benzaldehyde (15.93g, 0.15 mmol) and ethane-1,2-dithiol (14.10g, 0.15mmol) in $CHCl_3$ (600cm^3) at 0 ^{o}C. After leaving the mixture standing at 0 ^{o}C for 48hr, it was successively washed with ice cold aqueous KOH solution (7%, 2 x 200cm^3) and water (200cm^3). The extract was dried (Na_2SO_4) and the solvent distilled off to give dithiolane **111** (24.68g, 90%) as a colourless oil which crystallised as a white solid; mp 24 ^{o}C (from hexane) (lit.[127] 24-26 ^{o}C); δ_H (500 MHz, $CDCl_3$) 3.34-3.37 (2 H, m, CH_AH$_B$), 3.49-3.53 (2 H, m, CH$_A$$H_B$), 5.64 (1 H, s, 2-H), 7.25-7.33 (3 H, m, Ar), 7.52 (2 H, d, *J* 8.5, Ar); δ_C (125 MHz, $CDCl_3$) 40.23, 56.30, 127.95, 128.04, 128.49, 140.32; m/z (EI) 182 (M^+, 100%), 105 (M^+-C_6H_5, 24%), 77 (M^+-$C_3H_5S_2$, 43%).

5.4. Synthesis and biotransformation of racemic sulfoxides for kinetic resolution with DMSO reductases.

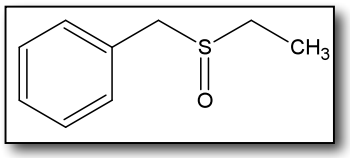
The crude mixture of bioproducts, obtained from each biotransformation, was purified by column chromatography and/or PLC. The residues were analysed, to determine the ee values of the metabolites, using the CSP HPLC systems mentioned in Chapter 2. The absolute configurations of the metabolites were asssigned by comparisons of their CSP HPLC profiles and/or optical rotation data with that of the enantiomers of known configurations.

5.4.1. Synthesis of dialkyl sulfides.



Benzylmethyl sulfoxide 70.

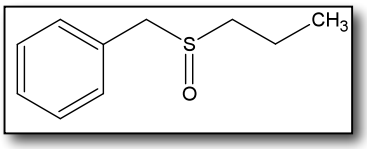
A solution of sodium periodate (9.30g, 43.5mmol) in water (100 cm^3) was added dropwise to a solution of benzyl methyl sulfide **81** (5g, 36.2mmol) in methanol (300cm^3), maintained at 0 ^oC. After leaving the reaction mixture stirred overnight, the solvent was evaporated off and the residue extracted with EtOAc. The extract was dried (Na_2SO_4) and the solvent removed to give the crude sulfoxide **70** (5.07g, 91%), which crystallised (from hexane) as colourless crystals; mp 50-54 ^oC (lit.[128] 52-55 ^oC, from $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.45 (3 H, s, Me), 3.92 (1 H, d, $J_{A,B}$ 12.9, CH_AH$_B$), 4.06 (1 H, d, $J_{B,A}$ 12.8, CH$_A$$H_B$), 7.28 (2 H, d, J 7.3, Ar), 7.35-7.39 (3 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 37.33, 60.41, 128.47, 129.01, 129.71, 130.03; m/z (EI) 154 (M^+, 25%).



Benzylethyl sulfoxide 71.

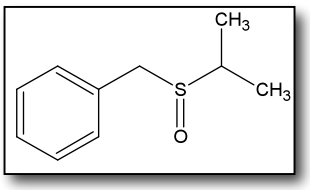
A solution of sodium periodate (9.30g, 43.5 mmol) in water (100cm^3) was added dropwise to a solution of benzylethyl sulfide **87** (5g, 36.2mmol) in methanol (300cm^3) kept at 0 ^oC. After leaving the reaction mixture stirred overnight, it was worked up as described for sulfoxide **70** to give the crude sulfoxide **71** (5.1g, 84%). It crystallised

as colourless crystals; mp 56-58 ^{o}C (from hexane) (lit.[129] 54-55 ^{o}C); δ_H (500 MHz, $CDCl_3$) 1.35 (3 H, t, *J* 7.5, Me), 2.53-2.68 (2 H, m, CH_2Me), 3.94 (1 H, d, $J_{A,B}$ 12.9, ArCH_AH$_B$), 4.03 (1 H, d, $J_{B,A}$ 12.9, ArCH$_A$$H_B$), 7.27-7.39 (5 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 6.61, 44.06, 57.58, 128.37, 129.01, 129.92, 129.98; m/z (EI) 168 (M^+, 27%).



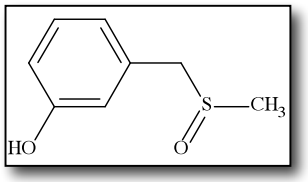
Benzylpropyl sulfoxide 72.

A solution of sodium periodate (1.9g, 9.0mmol) in water (25cm^3) was added dropwise to a solution of benzylpropyl sulfide **88** (1g, 6.0mmol) in methanol (50cm^3) maintained at 0 ^{o}C. After leaving the reaction mixture stirred overnight, it was worked up as described for sulfoxide **70** to give the crude sulfoxide **72** (0.98g, 90%) which crystallised as colourless crystals; mp 45 ^{o}C (from hexane) (lit.[128] 42-43 ^{o}C, from $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.03 (3 H, t, *J* 7.4, Me), 1.76-1.83 (2 H, m, CH_2Me), 2.52-2.59 (2 H, m, CH_2Et), 3.94 (1 H, d, $J_{A,B}$ 12.9, ArCH_AH$_B$), 4.03 (1 H, d, $J_{B,A}$ 12.9, ArCH$_A$$H_B$), 7.29 (2 H, d, *J* 8.1, Ar), 7.34-7.40 (3 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 13.38, 16.18, 52.90, 59.57, 128.33, 128.97, 130.00, 130.52; m/z (EI) 182 (M^+, 28%).



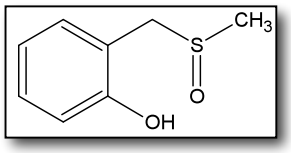
Benzyl *i*-propyl sulfoxide 73.

A solution of sodium periodate (1.9g, 8.9mmol) in water (25cm^3) was added dropwise to a solution of benzyl *iso*-propyl sulfide **89** (1g, 6.0mmol) in methanol (50cm^3) which was maintained at 0 ^{o}C. After leaving the reaction mixture overnight, it was worked up as described for sulfoxide **70** to give the crude sulfoxide **73** (0.93g, 85%) as a white crystalline solid; mp 36 ^{o}C (from hexane) (lit.[129] 25-27 ^{o}C); δ_H (500 MHz, $CDCl_3$) 1.28 (3 H, d, $J_{CH,MeA}$ 7.0, CMe_AMe$_B$), 1.33 (3 H, d, $J_{CH,MeB}$ 6.8, CMe$_A$$Me_B$), 2.65-2.71 (1 H, m, CH), 3.91 (2 H, m, CH_2), 7.30-7.37 (5 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 13.78, 16.70, 48.25, 55.01, 128.20, 128.96, 129.92, 130.58; m/z (EI) 182 M^+, 77%).



(3-hydroxybenzyl)methyl sulfoxide 74.

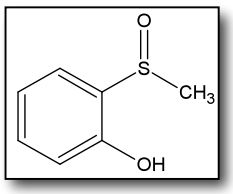
Phenol **74** was the major component, obtained as a white crystalline solid (67% relative yield), from the decomposition of diol sulfoxide **125**, mp 120 ^{o}C (from $CHCl_3$); R_f 0.35 (7% MeOH/$CHCl_3$); (Found: M^+, 170.0402; $C_8H_{10}O_2S$ requires 170.0397); δ_H (500 MHz, $CDCl_3$) 2.50 (3 H, s, CH_3), 3.80 (1 H, d, $J_{A,B}$ 14.2, ArCH_AH$_B$), 4.46 (1 H, d, $J_{B,A}$ 14.2, ArCH$_A$$H_B$), 6.90 (1 H, m, Ar), 7.01-7.05 (2 H, m, Ar), 7.26 (1 H, m, Ar), 9.17 (1 H, s, O*H*); δ_C (125 MHz, $CDCl_3$) 35.89, 55.18, 117.73, 119.33, 120.61, 130.64, 132.22, 156.80; m/z (EI) 170 (M^+, 35%), 107 (M^+-CH_3SO, 100%).



(2-hydroxybenzyl)methyl sulfoxide 84.

Phenol **84**, a white crystalline solid (33% relative yield); was obtained as a minor component, from the decomposition of diol sulfoxide **125**, mp 116 ^{o}C (from EtOAc) (lit.[130] 108-110^{o}C); δ_H (500 MHz, $CDCl_3$) 2.52 (3 H, s, Me), 3.93 (1 H, d, $J_{A,B}$ 13.7, CH_AH$_B$), 4.35 (1 H, d, $J_{B,A}$ 13.7, CH$_A$$H_B$), 6.90 (1 H, m, Ar), 3.99 (1 H, d, *J* 8.2 Ar), 7.09 (1 H, d, *J* 7.5, Ar), 7.23 (1 H, m, Ar), 9.08 (1 H, s, O*H*); δ_C (125 MHz, $CDCl_3$) 36.18, 54.88, 117.09, 118.11, 120.39, 130.40, 132.14, 156.58; m/z (EI) 170 (M^+, 20%), 107 (M^+-CH_3SO, 100%).

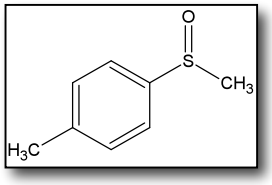
5.4.2. Synthesis of dialkyl sulfides.



2-(Methylsulfinyl)phenol 77.

A stirred solution of 2-(methylsulfanyl)phenol (5g, 35.7mmol), in acetone (50cm^3) maintained at 0 ^{o}C, was oxidised by the dropwise addition of a solution of DMD in acetone. The progress of oxidation reaction was followed by TLC analysis of the reaction mixture. After completion of the reaction acetone was evaporated off and the

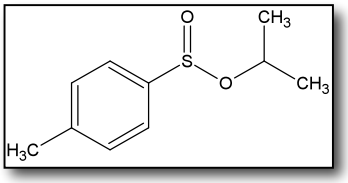
residue dried (vaccum pump). The crude dried sample was crystallised, to furnish sulfoxide **77** (5.30g, 95%) as a colourless crystalline solid; mp 123 ^{o}C (from MeOH) (lit.[131] 127-128 ^{o}C); R_f 0.23 (5% MeOH/$CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.96 (3 H, s, Me), 6.93 (1 H, m, Ar), 6.97 (1 H, d, *J* 7.7, Ar), 7.07 (1 H, d, *J* 7.8, Ar), 7.37 (1 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 41.91, 119.71, 119.86, 123.07, 124.83, 133.05; *m/z* (EI) 156 (M^+, 100%), 141 (M^+-CH_3, 95%). Sulfoxide **77** (0.5g), was used as a substrate with *Citronella braakwi* DMSO-11. The progress of the biotransformation was monitored by RP HPLC (30% H_2O/MeOH). The biotransformation was terminated when 55% of the substrate **77** had metabolised to the corresponding sulfide. The aqueous biotransformation solution was concentrated under reduced pressure and the residue extracted with EtOAc. The crude material, obtained after removal of the solvent, was purified by PLC (5% MeOH/$CHCl_3$) to yield sulfoxide **77** (135mg, 27%); ($[\alpha]_D$) of – 189 (*c* 0.5, $CHCl_3$) (lit.[101] – 191, $CHCl_3$);The enantiomeric excess (≥ 98%) was determined by CSP HPLC analysis using a Chiralcel OB column (10% IPA/hexane, flow 0.5 cm^3/min, α 1.58); CD: λ 281nm $\Delta\varepsilon$ - 5.93, λ 236nm $\Delta\varepsilon$ - 1.50, λ 215nm $\Delta\varepsilon$ 6.39, λ 203nm $\Delta\varepsilon$ – 6.15.



Methyl *p*-tolyl sulfoxide 29.

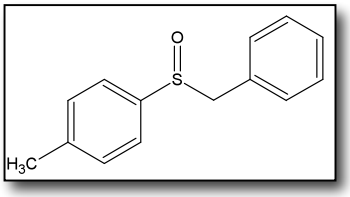
A solution of sodium periodate (4.65g, 21.7mmol) in water (50cm^3) was added dropwise to a solution of methyl-*p*-tolyl sulfide **79** (2g, 14.5mmol) in methanol (75cm^3) maintained at 0^{o}C; the reaction mixture was left stirring overnight and then worked up by the procedure described for sulfoxide **70** to give the crude sulfoxide **29** (2.01g, 90%) as a white crystalline solid; mp 45-48 ^{o}C (from hexane) (lit.[132] 41-42 ^{o}C); R_f 0.24 (Et_2O); δ_H (500MHz, $CDCl_3$) 2.41 (3 H, s, Ar*Me*), 2.71 (3 H, s, SO*Me*), 7.33 (2 H, d, *J* 8.0, Ar), 7.54 (2 H, d, *J* 8.1, Ar); δ_C (125 MHz, $CDCl_3$) 21.40, 43.99, 123.57, 130.06, 141.55, 142.52; *m/z* (EI) 154 (M^+, 91%), 139 (M^+-CH_3, 100%), 91 (M^+-CH_3SO).

5.4.3. Synthesis of benzyl *para*-tolyl sulfoxide 91.



***i*-Propyl *p*-toluene sulfinate 92.**

p-Toluenesulfinic acid, sodium salt (4g, 22.5mmol) was suspended in thionyl chloride (15cm^3) and the mixture stirred in a partially stoppered flask for 30min. Excess of thionyl chloride was distilled off and anhydrous *iso*-propanol (40 cm^3) was added to the residue followed by Et_3N (10 cm^3). Water (75 cm^3) was then added, the crude product extracted with EtOAc from the quenched reaction mixture and dried (Na_2SO_4). The solvent was distilled off leaving sulfinate **92** (4.40g, 98%) as a colourless oil which decomposed on distillation; R_f 0.84 (Et_2O); δ_H (500 MHz, $CDCl_3$) 1.24 (3 H, d, $J_{CH,MeA}$ 6.3, CH*Me*$_A$Me$_B$), 1.38 (3 H, d, $J_{CH,MeB}$ 6.2, CHMe$_A$*Me*$_B$), 2.42 (3 H, s, Ar*Me*), 4.57-4.62 (1 H, m, CH), 7.32 (2 H, d, *J* 8.3, Ar), 7.60 (2 H, d, *J* 8.2, Ar); δ_C (125 MHz, $CDCl_3$) 21.50, 23.76, 23.96, 72.65, 125.06, 129.61, 142.47, 142.79; m/z (EI) 155 (M^+-C_3H_7, 13%), 107 (M^+-C_7H_7, 77%), 91 (M^+-$C_3H_7O_2S$, 82%). The spectral data of sulfinate **92** was identical with the literature data.[133]

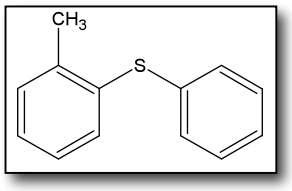


Benzyl *p*-tolyl sulfoxide 91.

Benzyl bromide **94** (1g, 5.85 mmol) was added to anhydrous ether (20 cm^3) containing dry magnesium turnings (0.2g, 8.33 mmol) under nitrogen gas atmosphere. After initiation of the reaction, the mixture was left stirring for 30 min. at ambient temperature. *iso*-Propyl-4-methyl-1-benzene sulfinate **92** (1.16g, 5.85mmol) was added dropwise into the reaction mixture; it was stirred for a further 15min. before quenching it by the addition of HCl solution (50 cm^3, 3M). The crude product was extracted with EtOAc dried (Na_2SO_4) and the solvent evaporated leaving sulfoxide **91** (1.14g, 97%) as a white crystalline solid; mp 140 ^{o}C (from MeOH) (lit.[134] 136-137 ^{o}C); R_f 0.51 (Et_2O); δ_H (500 MHz, $CDCl_3$) 2.40 (3 H, s, Me), 3.97 (1

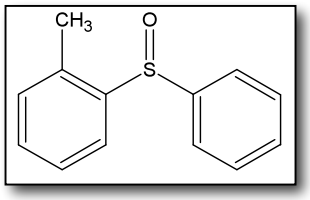
H, d, $J_{A,B}$ 12.6, CH_AH$_B$), 4.10 (1 H, d, $J_{B,A}$ 12.6, CH$_A$$H_B$), 6.99 (2 H, d, *J* 7.6, Ar), 7.21-7.28 (7 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 21.46, 63.67, 124.48, 128.19, 128.45, 129.29, 129.56, 130.37, 139.47, 141.65; m/z (EI) 230 (M^+, 20%), 214 (M^+-H_2O, 65%), 139 (M^+-C_7H_7, 84%), 91 (M^+-C_7H_7OS, 63%), 77 (M^+-C_8H_9OS, 100%).

5.4.4. Synthesis of Phenyl *o*-tolyl sulfoxide 78 for kinetic resolution.



Phenyl *o*-tolyl sulfide 83.

The Grignard's reagent of *o*-bromo toluene **102** (5g, 29.2mmol) was prepared by its slow addition to a mixture of magnesium filings (0.84g, 35mmol) in anhydrous ether (100 cm^3) under an inert atmosphere. Diphenyldisulfide (6.3g, 29mmol), in ether (20 cm^3), was added dropwise and the reaction mixture refluxed (1hr) befor quenching with the addition of HCl solution (50 cm^3, 3M). The ether layer was separated, washed with potassium carbonate solution (50 cm^3, 1M), dried (Na_2SO_4) and evaporated to give the crude sulfide product. It was purified by column chromatography (hexane) to furnish a pure sample of sulfide **83** (4.99 g, 86%) as a colourless oil; bp 86-88^{o}C /0.1mmHg (lit.[135] 170^{o}C /22mmHg); R_f 0.46 (hexane); δ_H (500 MHz, $CDCl_3$) 2.38 (3 H, s, Me), 7.10-7.27 (9 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 20.58, 126.33, 126.71, 127.90, 129.12, 129.64, 130.60, 133.02, 133.77, 136.18, 140.00; m/z (EI) 200 (M^+, 100%), 91 (M^+-C_6H_6S, 16%).

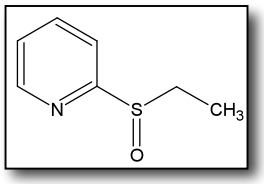


Phenyl *o*-tolyl sulfoxide 78.

Phenyl *o*-tolyl sulfide (4g, 20mmol) was oxidised, following the procedure described for the oxidation of sulfide **81**, to give a the crude sulfoxide. It was purified by column chromatography (Et_2O) to furnish sulfoxide **78** (3.24g, 75%) as a crystalline solid; mp 42 ^{o}C (from MeOH) (lit[136] 42 ^{o}C); R_f 0.73 (Et_2O); δ_H (500 MHz, $CDCl_3$) 2.36 (3 H, s, Me), 7.16 (1 H, d, *J* 7.4, Ar), 7.36-7.44 (5 H, m, Ar), 7.59 (2 H, m, Ar),

7.94 (1 H, d, *J* 7.7, Ar); δ_C (125 MHz, $CDCl_3$) 18.60, 124.77, 125.87, 125.91, 127.15, 129.31, 130.99, 131.09, 135.79, 142.98, 144.64; m/z (EI) 216 M^+, 70%), 91 (M^+-C_6H_6SO, 68%), 77 (M^+-C_7H_7SO, 93%)

5.4.5. Synthesis of Ethyl-(2-pyridyl) sulfoxide 75.

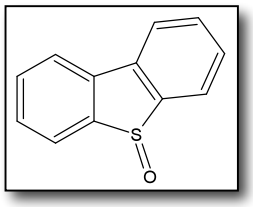


Ethyl-2-pyridyl sulfoxide 75.

Ethyl-2-pyridyl sulfide **80** (3g, 21.6mmol) was oxidised, following the procedure described for the oxidation of sulfide **81**, to give the crude sulfoxide. It was purified by column chromatography (5% MeOH/$CHCl_3$) to yield sulfoxide **75** (2.71g, 81%) as a colourless oil; bp 120°C /4mmHg (lit.[137] 123°C /4mmHg); δ_H (500 MHz, $CDCl_3$) 1.21 (3 H, m, Me), 2.90-2.97 (1 H, m, CH_AH$_B$), 3.15-3.22 (1 H, m, CH$_A$$H_B$), 7.38 (1 H, d, *J* 7.4, Ar), 7.92-8.00 (2 H, m, Ar), 8.63 (1 H, d, J 4.8, Ar); δ_C (125 MHz, $CDCl_3$) 5.40, 47.44, 120.40, 124.46, 137.78, 149.62, 164.11. The aqueous mixture of bioproducts, obtained when sulfoxide **75** (2.0g) was metabolised with *P. vulgaris*, was extracted with EtOAc after removal of most the water under reduced pressure. The residue, left after removal of the solvent from the extract, was purified by PLC to yield (-)-(*S*)-ethyl-2-pyridyl sulfoxide **75** (0.90g, 45%); $[\alpha]_D$ - 157 (*c* 0.6, $CHCl_3$) (lit.[138] - 168, MeOH). The enantiomeric excess (≥ 98%) of sulfoxide **75** was determined by CSP HPLC analysis using a Chiralcel OB column (10% IPA/hexane, flow 0.5 cm^3/min, α 1.95).

5.5. Synthesis of thiophene sulfoxides and a sulfinate for kinetic resolution with DMSO reductases.

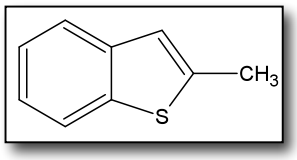
5.5.1. Synthesis of Dibenzo[*b*]thiophene sulfoxide 97.



Dibenzo[*b*]thiophene sulfoxide 97.

A solution of *M*CPBA (0.94g, 5.4mmol) in CH_2Cl_2 (50 cm^3) was added dropwise (30min.) to a mixture of dibenzothiophene (1g, 5.4mmol) and BF_3-Et_2O (6.8ml, 54mmol) in CH_2Cl_2 (50 cm^3) under N_2 gas atmosphere at – 20 ^oC. The reaction mixture was stirred (4hr) and then allowed to warm up to room temp; it was washed with cold water, a saturated solution of $NaHCO_3$ (50 cm^3), filtered through celite, dried (Na_2SO_4) and the solvent evaporated off. The crude sulfoxide **97** (1.03g, 96%) thus obtained was crystallized as a white solid; mp 190-193 ^oC (from Et_2O) (lit.[139] 189-191 ^oC); δ_H (500 MHz, $CDCl_3$) 7.51 (2 H, m, Ar), 7.59 (2 H, m, Ar), 7.80 (2 H, d, *J* 7.7, Ar), 7.98 (2 H, d, *J* 7.6, Ar); δ_C (125 MHz, $CDCl_3$) 121.91, 127.54, 129.55, 132.55, 137.10, 145.17; m/z (ES) 223 ($[M+23]^+$, 100%), 201 ($[M+1]^+$, 73%).

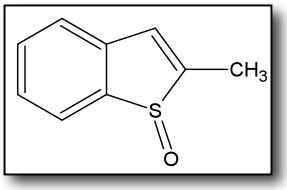
5.5.2. Synthesis of 2-substituted Benzo[*b*]thiophene sulfoxides.



2-Methylbenzo[*b*]thiophene 98.

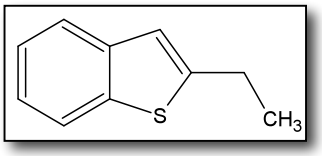
t-Butyl lithium in hexane (13.2 cm^3, 33mmol, 2.5M), was added slowly (0.5hr) to a solution of benzo[*b*]thiophene (4.4g, 33mol) in dry THF (50 cm^3) under an argon atmosphere at – 70 ^oC. After stirring (0.5hr) methyl iodide (6.0g, 42.3mmol) was added in one portion to the reaction mixture. It was stirred for a further period of one hour and then ammonium chloride solution (50 cm^3, 2M) was added to quench the reaction. Most of the THF was removed by distillation under reduced pressure, the remaining aqueous portion extracted with ether (2 x 75 cm^3) and dried (Na_2SO_4). The crude product obtained, after evaporation of ether, was purified by column

chromatography (hexane) to yield thiophene **98** (4.8g, 94%) as a white crystalline solid.; mp 48°C (from hexane) (lit.[140] 51-52°C); δ_H (500 MHz, $CDCl_3$) 2.58 (3 H, s, Me), 6.97 (1 H, s, 3-H), 7.23-7.39 (2 H, m, Ar), 7.64 (1 H, d, *J* 7.7, Ar), 7.73 (1 H, d, *J* 8.0 Ar); δ_C (125 MHz, $CDCl_3$) 16.14, 121.59, 121.99, 122.52, 123.34, 124.05, 139.69, 140.47, 140.85; m/z (EI) 148 (M^+, 85%).



2-Methylbenzo[*b*]thiophene-1-oxide 96.

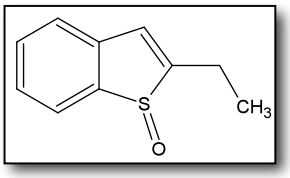
A solution of *M*CPBA (4.8g, 30.4 mmol) in dry CH_2Cl_2 (100 cm^3) was added dropwise to a solution of thiophene **98** (4.5g, 30.4mol) in CH_2Cl_2 (100 cm^3, dry) and BF_3-Et_2O (40g, 34 cm^3, 0.28mol), under N_2 gas atmosphere, at – 20 °C. The reaction mixture was stirred (4hr) and then allowed to warm up to room temp; it was washed with cold water, a saturated solution of $NaHCO_3$ (50 cm^3), filtered through celite, dried (Na_2SO_4) and the solvent evaporated off. The sulfoxide **96** (4.54g, 91%) was obtained a crystalline solid; mp 74-75 °C (from Et_2O) (lit.[141] 80-81 °C); R_f 0.51 (4% MeOH/$CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.39 (3 H, s, Me), 6.78 (1 H, s, 3-H), 7.36 (2 H, m, Ar), 7.46 (1 H, m, Ar), 7.58 (1 H, d, *J* 7.6, Ar); δ_C (125 MHz, $CDCl_3$) 13.02, 123.62, 126.25, 127.65, 128.68, 132.02, 138.16, 144.64, 150.51; m/z (EI) 164 (M^+, 86%), 149 (M^+-CH_3, 100%).



2-Ethylbenzo[*b*]thiophene 99.

t-Butyl lithium in hexane (13.2 cm^3, 33mmol, 2.5M), was added slowly (30 min) to a solution of benzo[*b*]thiophene (4.4g, 33mol), in anhydrous THF (50 cm^3) under an argon atmosphere at – 70 °C. It was stirred for a further period of one hour and ammonium chloride solution (50 cm^3, 2M) was then added to quench the reaction. Most of the THF was removed by distillation under reduced pressure, the remaining aqueous portion extracted with Et_2O (2 x 75 cm^3) and dried (Na_2SO_4). Purification, of the crude product, obtained after removal of Et_2O, by column chromatography (hexane), gave thiophene **99** (4.8g, 91%) as a colourless oil; bp 130°C /15mmHg

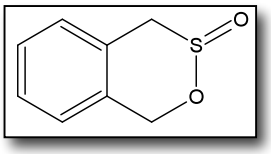
(lit.[142] 128°C /15mmHg); δ_H (500 MHz, $CDCl_3$) 1.37 (3 H, t, *J* 7.5, Me), 2.92 (2 H, q, *J* 7.5, CH_2), 6.99 (1 H, s, 3-H), 7.21-7.31 (2 H, m, Ar), 7.65 (1 H, d, *J* 7.8, Ar), 7.75 (1 H, d, *J* 8.0 Ar); δ_C (125 MHz, $CDCl_3$) 15.44, 24.12, 119.66, 122.12, 122.68, 123.35, 124.03, 139.22, 140.26, 148.32; m/z (EI) 162 (M^+, 65%), 147 (M^+-CH_3, 100%).



2-Ethylbenzo[*b*]thiophene-1-oxide 95.

The thiophene **98** (4.5g, 27.8mol) was oxidised with *M*CPBA (4.8g, 27.8mmol) in the presence of BF_3-Et_2O (4 g, 34 cm^3, 0.28mol) following the procedure described for the oxidation of thiophene **96**. Purification of the crude product by column chromatography (5% MeOH/$CHCl_3$) gave thiophene oxide **95** (4.55g, 92%) as a colourless oil; mp 55°C (from hexane); R_f 0.55 (4% MeOH/$CHCl_3$); (Found: M^+, 178.2478; $C_{10}H_{10}OS$ requires 178.2481); δ_H (500 MHz, $CDCl_3$) 1.36 (3 H, t, *J* 7.4, Me), 2.71-2.85 (2 H, m, CH_2), 6.76 (1 H, s, 3-H), 7.35-7.38 (2 H, m, Ar), 7.45 (1 H, d, *J* 7.5, Ar), 7.86 (1 H, d, *J* 4.8, Ar); δ_C (125 MHz, $CDCl_3$) 13.30, 21.17, 124.14, 126.66, 127.30, 128.04, 132.41, 138.50, 144.73, 157.12; m/z (EI) 178 M^+, 74%), 163 (M^+-CH_3, 96%), 149 (M^+-C_2H_5, 92%).

5.6. Synthesis of benzo[*d*]-1,2-oxathiane-2-oxide 101 for kinetic resolution.



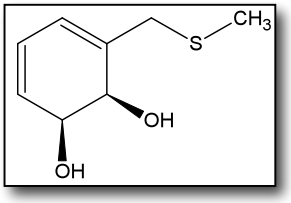
Benzo[*d*]-1,2-oxathiane-2-oxide 101.

Sodium hydroxymethyl sulfinate dihydrate (16.4g, 0.107mol) was added to an ice cold solution of 1,2-di(bromomethyl)benzene (14.0g, 53.2mmol) and tetrabutyl ammonium bromide (3.43g) in dry DMF (110 cm^3). After stirring the reaction mixture at 0 °C for 7hr and at room temp for another 7hr, water (200 cm^3) was added and the mixture extracted with Et_2O (4 × 100 cm^3) and the Et_2O extract washed with water (2 × 75 cm^3). Ether was removed from the dried extract (Na_2SO_4) and the residue purified by column chromatography (ether) to yield oxathiane oxide **101**

(6.0g, 67%) as a white crystalline solid; mp 51-54 ^{o}C (from EtOAc/hexane); R_f 0.73 (ether); δ_H (500 MHz, $CDCl_3$) 3.56 (1 H, d, $J_{A,B}$ 15.4, OCH_AH$_B$), 4.43 (1 H, d, $J_{B,A}$ 15.4, OCH$_A$$H_B$), 4.97 (1 H, d, $J_{A,B}$ 13.7, SOCH_AH$_B$), 5.31 (1 H, d, $J_{B,A}$ 13.7, SOCH$_A$$H_B$), 7.22-7.27 (2 H, m, Ar), 7.34-7.37 (2 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 56.42, 62.44, 125.17, 125.62, 127.33, 128.08, 129.10, 133.19; m/z (EI) 168 (M^+, 7%), 104 (M^+-SO_2, 90%), (ES) 191 ([M+23]$^+$, 51%), 169 ([M+1]$^+$, 100%). The spectral data of oxathiane oxide **101** was identical to the literature data[143] which was reported as an oil.

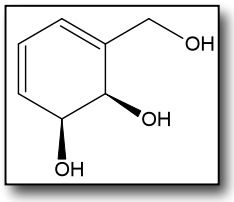
5.7. Biotransformation of benzylmethyl sulfide 81 with *P. putida* UV4.

The biotransformation media, from the biotransformation of commercially available benzylmethylsulfide **81** (5g, 36.23mmol), was evaporated and the viscous concentrate repeatedly extracted with EtOAc. The crude evaporated extract-residue was purified by column chromatogrpahy followed by PLC of the selected column fractions;
the following metabolites were isolated:



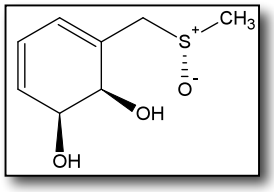
(+)-*cis*-(1*S*,2*R*)-3-(Methylsulfanylmethyl)-3,5-cyclohexadiene-1,2-diol 85.

Isolated by column chromatogrpahy (4% MeOH/$CHCl_3$), sulfide diol **85** (2.52g, 35%) was obtained as a white crystalline solid; mp 67^{o}C (from Et_2O/hexane, decomp.); R_f 0.43 (10% MeOH/$CHCl_3$); $[\alpha]_D$ + 131 (*c* 1.0 in $CHCl_3$); (Found: C, 56.1; H, 7.1. $C_8H_{12}O_2S$ requires C, 55.8; H, 7.0 %); δ_H (500 MHz, $CDCl_3$) 2.03 (3 H, s, Me), 2.19 (1 H, d, *J* 8.1, OH), 2.56 (1 H, d, *J* 7.2, OH), 3.27 (1 H, d, $J_{A,B}$ 13.5, CH_AH$_B$), 3.32 (1 H, d, $J_{B,A}$ 13.6, CH$_A$$H_B$), 4.30 (1 H, d, $J_{1,2}$ 6.0, 2-H), 4.34 (1 H, m, 1-H), 5.78 (1 H, d, *J* 4.9, 4-H), 5.91 (1 H, dd, *J* 9.3, 3.1, 6-H), 5.96-5.99 (1 H, m, 5-H); δ_C (125 MHz, $CDCl_3$) 14.52, 37.63, 68.57, 69.64, 122.01, 124.44, 129.30, 136.45; m/z (EI) 154 (M^+-H_2O, 19%), (ES) 195 ([M+23]$^+$, 100%); CD: λ 274nm Δε 5.14, λ 238nm Δε 1.35, λ 230nm Δε 1.82, λ 209nm Δε -1.75.



(+)-*cis*-(1S,2R)-3-Hydroxymethylcyclohexa-3,5-diene-1,2-diol 86.

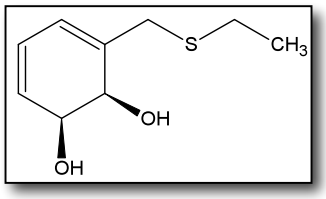
Isolated, by a combination of column chromatography (4% MeOH/$CHCl_3$) and multiple elution PLC (7% MeOH/$CHCl_3$) triol **86** (56mg, 0.9%) was found to be a colourless oil which decomposed on heating; R_f 0.25 (12% MeOH/$CHCl_3$); $[\alpha]_D$ + 33.6 (*c* 0.5, MeOH); (Found: M^+-H_2O, 124.0525. $C_7H_8O_2$ requires 124.0524); δ_H (500 MHz, $CDCl_3$) 4.28 (1 H, m, 1-H), 4.31 (2 H, s, CH_2), 4.38 (1 H, d, $J_{1,2}$ 6.0, 2-H), 5.93 (3 H, m, 4- & 6-H), 6.01 (1 H, dd, *J* 5.1, 9.4, 5-H); δ_C (125 MHz, $CDCl_3$) 62.55, 65.94, 67.65, 118.69, 122.97, 126.96, 137.54; m/z (EI) 142 (M^+, 0.5%), 124 (M^+-H_2O, 10%). The spectral data of diol **86** was identical to the literature data.[113]



(+)-*cis*-(1*S*,2*R*)-3-(*S*-Methylsulfinylmethyl)-3,5-cyclohexadiene-1,2-diol 122.

Sulfoxide diol **122** (66mg, 0.8%) was isolated as colourless oil; R_f 0.23 (10% MeOH/$CHCl_3$); $[\alpha]_D$ + 71.8 (*c* 0.9, $CHCl_3$); (Found: M^+-H_2O, 170.0402. $C_8H_{10}O_2S$ requires 170.0406); δ_H (500 MHz, $CDCl_3$) 2.58 (3 H, s, Me), 3.53 (1 H, d, $J_{A,B}$ 13.0, CH_AH$_B$), 3.88 (1 H, d, $J_{B,A}$ 13.0, CH$_A$$H_B$), 4.27 (1 H, d, $J_{1,2}$ 6.6, 2-H), 4.30 (1 H, m, 1-H), 6.02 (1 H, m, 4-H), 6.07 (2 H, m, 5- & 6-H); δ_C (125 MHz, $CDCl_3$) 37.65, 57.71, 67.60, 70.33, 125.04, 126.70, 130.10, 132.18; m/z (EI) 170 (M^+-H_2O, 37 %); CD: λ 269nm $\Delta\varepsilon$ 7.82, λ 238nm $\Delta\varepsilon$ - 5.98, λ 218nm $\Delta\varepsilon$ 1.25, λ 203nm $\Delta\varepsilon$ - 4.76.

5.8. Biotransformation of benzylethyl sulfide 87 with *P. putida* UV4.

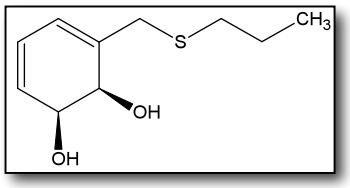


(+)-*cis*-(1*S*,2*R*)-3-(Ethylsulfanylmethyl)-3,5-cyclohexadiene-1,2-diol 123.

Biotransformation of a commercially available sample of benzylethyl sulfide **87** (20g, 131.6mmol), followed by the usual work up of the aqueous biotransformed material

and column chromatogrpahy (4% MeOH/$CHCl_3$) of the crude product, yielded diol **123** (2.54g, 21%) as a white crystalline solid; mp 48 ^oC (from Et_2O/hexane); $[\alpha]_D$ + 69.4 (*c* 0.5, $CHCl_3$); (Found: C, 58.0. H, 7.7; $C_9H_{14}O_2S$ requires C, 58.0. H, 7.6%); (Found: M^+, 186.0710. $C_9H_{14}O_2S$ requires 186.0715); (Found: M^+-H_2O, 168.0601. $C_9H_{12}OS$ requires 168.0609); δ_H (500 MHz, $CDCl_3$) 1.24 (3 H, t, *J* 7.4, Me), 2.48 (2 H, q, *J* 7.4, CH_2Me), 3.33 (1 H, d, $J_{A,B}$ 13.7, CH_AH$_B$SEt), 3.36 (1 H, d, $J_{B,A}$ 13.5, CH$_A$$H_B$SEt), 4.30 (1 H, d, $J_{1,2}$ 6.0, 2-H), 4.32 (1 H, m, 1-H), 5.78 (1 H, d, *J* 5.2, 4-H), 5.89 (1 H, dd, *J* 9.6, *J* 3.1, 6-H), 5.95 (1 H, dd, *J* 9.6, *J* 5.2, 5-H); δ_C (125 MHz, $CDCl_3$) 14.69, 25.27, 35.47, 68.97, 69.95, 122.20, 124.80, 129.62, 136.85; m/z (EI) 186 (M^+, 6%), 168 (M^+-H_2O, 77%), 29 (M^+-$C_7H_9O_2S$, 100%); CD: λ 275nm $\Delta\varepsilon$ 6.51, λ 229nm $\Delta\varepsilon$ 2.28.

5.9. Biotransformation of benzylpropyl sulfide 88 with *P. putida* UV4.

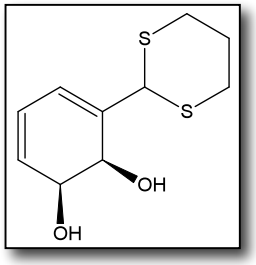


(+)-*cis*-(1*S*,2*R*)-3-(Propylsulfanylmethyl)-3,5-cyclohexadiene-1,2-diol 124.

Biotransformation of a commercially available benzylpropylsulfide **88** (20g, 120.5mmol) and purification of the crude bioproduct by column chromatogrpahy (4% MeOH/$CHCl_3$) gave diol **124** (1.43g, 10%) as a colourless oil which decomposed on heating; $[\alpha]_D$ + 72.5 (*c* 0.4, $CHCl_3$); (Found: M^+, 200.0871. $C_{10}H_{16}O_2S$ requires 200.0871); δ_H (500 MHz, $CDCl_3$) 0.97 (3 H, t, *J* 7.3, Me), 1.55-1.63 (2 H, m, CH_2Me), 2.43 (2 H, t, *J* 7.2, CH_2Et), 3.31 (1 H, d, $J_{A,B}$ 13.5, CH_AH$_B$SPr), 3.35 (1 H, d, $J_{B,A}$ 13.5, CH$_A$$H_B$SPr), 4.30 (1 H, d, $J_{1,2}$ 6.1, 2-H), 4.31 (1 H, m, 1-H), 5.78 (1 H, d, *J* 5.0, 4-H), 5.88 (1 H, dd, *J* 9.6, *J* 2.8, 6-H), 5.95 (1 H, dd, *J* 9.6, *J* 5.2, 5-H); δ_C (125 MHz, $CDCl_3$) 13.85, 22.85, 33.44, 35.84, 69.05, 122.23, 124.74, 129.62, 136.90; m/z (EI) 200 (M^+, 6%), 182 (M^+-H_2O, 72%); CD: λ 272nm $\Delta\varepsilon$ 2.71, λ 237nm $\Delta\varepsilon$ 0.41, λ 229nm $\Delta\varepsilon$ 0.71, λ 209 $\Delta\varepsilon$ - 0.38.

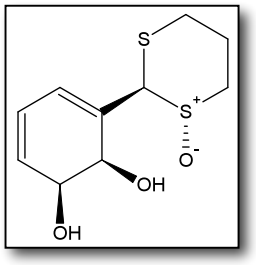
5.10. Biotransformation of 2-phenyl-1,3-dithiane 110 with *P. putida* UV4.

Biotransformation of the commercially available **129** (7.9g, 40.36mmol) and purification of the crude bioproduct mixture, by column chromatogrpahy (5% MeOH/$CHCl_3$), furnished three compounds; diols **129** and **130** and dithiane oxide **128**.



(+)-*cis*-(1*S*,2*R*)-3-(1',3'-Dithian-2'-yl)-3,5-cyclohexadiene-1,2-diol 129.

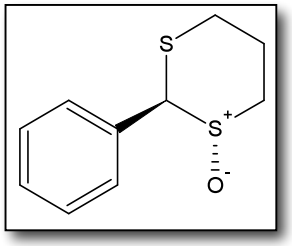
129 (1.67g, 18%) colourless solid; mp 51-53 ^oC (from Et_2O/hexane); R_f 0.31 (5% MeOH/$CHCl_3$); $[\alpha]_D$ + 81.5 (*c* 0.7, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.84-1.93 (1 H, m, 5'-CH_AH$_B$), 2.11-2.17 (1 H, m, 5'-CH$_A$$H_B$), 2.82-3.01 (6 H, m, SCH_2 + OH), 4.29 (1 H, d, $J_{1,2}$ 6.4, 2-H), 4.33 (1 H, m, 1-H), 4.88 (1 H, s, 2'-H), 5.90 (1 H, dd, *J* 9.6, 3.3, 6-H), 5.99 (1 H, dd, *J* 9.6, 5.5, 5-H), 6.18 (1 H, d, *J* 5.3, 4-H); δ_C (125 MHz, $CDCl_3$) 25.89, 31.87, 32.01, 49.05, 69.20, 70.10, 124.21, 124.56, 131.11, 138.77; m/z (EI) 230 M^+, 94%), 212 (M^+-H_2O, 96%); CD: λ 276nm $\Delta\varepsilon$ 3.55, λ 254nm $\Delta\varepsilon$ 0.94, λ 242nm $\Delta\varepsilon$ 2.61, λ 214nm $\Delta\varepsilon$ - 3.05.



(+)-*cis*-(*1S,2R*)-3-(*trans*-(*1'S,2'S*)-1',3'-Dithan-2'yl-1'-oxy)cyclohexa-3,5-diene-1,2-diol 130.

130 (1.19g, 12%) white crystalline solid; mp 112 ^oC (from Acetone/ Et_2O); R_f 0.17 (5%MeOH/$CHCl_3$); $[\alpha]_D$ + 48.7 (*c* 1.0, $CHCl_3$), + 150 (*c* 0.6, H_2O); (Found: M^+ C, 48.8. H, 5.6. $C_{10}H_{14}O_3S_2$ requires C, 48.8. H, 5.7%); (Found: M^+, 246.0386. $C_{10}H_{14}O_3S_2$ requires 246.0385); δ_H (500 MHz, $CDCl_3$) 2.24-2.33 (1 H, m, 5'-CH_AH$_B$), 2.48-2.53 (1 H, m, 5'-CH$_A$$H_B$), 2.65-2.70 (1 H, m, 4'-CH_AH$_B$), 2.77-2.86 (2 H, m, 4'-CH$_A$$H_B$ & 6'-CH_AH$_B$), 3.29 (1 H, m, 1-OH), 3.52 (1 H, d, *J* 12.8, 6'-CH$_A$$H_B$), 4.24 (1 H, d, $J_{1,2}$ 5.5, 2-H), 4.30 (1 H, m, 1-H), 4.43 (1 H, d, *J* 6.1, 2-OH), 4.61 (1 H, s, 2'-H), 5.99-6.07 (2 H, m, 5- & 6-H), 6.14 (1 H, d, *J* 5.2, 4-H); δ_C (125 MHz, $CDCl_3$) 29.55,

31.26, 54.41, 65.63, 67.98, 69.27, 123.32, 125.91, 131.95, 134.33; m/z (EI) 246 (M^+, 91%), 228 (M^+-H_2O, 55%); CD: λ 280nm $\Delta\varepsilon$ 4.25, λ 227nm $\Delta\varepsilon$ - 6.37.

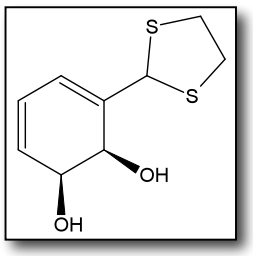


(+)-*trans*-(*1S,2S*)-2-Phenyl-1,3-dithiane-1-oxide 128.

128 (0.639g, 7%) colourless crystals; mp 155-157 ^oC (from $CHCl_3$) (lit.[137] 145-147^oC, racemic); R_f 0.37 (5%MeOH/$CHCl_3$); $[\alpha]_D$ + 90.9 (*c* 1.2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.28-2.38 (1 H, m, 5-CH_AH$_B$), 2.45-2.50 (1 H, m, 5-CH$_A$$H_B$), 2.62-2.67 (1 H, m, 4-CH_AH$_B$), 2.70-2.77 (1 H, 6-CH_AH$_B$), 2.80-2.88 (1 H, m, 4-CH$_A$$H_B$), 3.50-3.57 (1 H, m, 6-CH$_A$$H_B$), 4.55 (1 H, s, 2-H), 7.26-7.45 (5 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 29.52, 31.41, 54.79, 69.68, 128.75, 129.11, 129.33, 133.39; m/z (EI) 212 (M^+, 100%), 135 (M^+-C_6H_5, 79%), 77 (M^+-$C_4H_7OS_2$, 66%); CD: λ 248nm $\Delta\varepsilon$ 1.60, λ 227nm $\Delta\varepsilon$ 6.12, λ 211nm $\Delta\varepsilon$ - 3.17.

5.11. Biotransformation of 2-phenyl-1,3-dithiolane 111 by *P. putida* UV4.

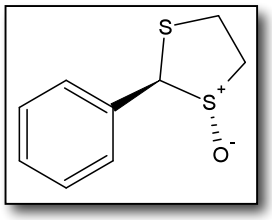
Biotransformation of the commercially available **111** (14.0g, 76.1mmol) and purification of the crude bioproduct mixture, by column chromatogrpahy (5% MeOH/$CHCl_3$), yielded three compounds; diols **135** and **141** and dithiolane oxide **134**:



(+)-*cis*-(*1S,2R*)-3-(1',3'-Dithiolan-2'-yl)cyclohexa-3,5-diene-1,2-diol 135.

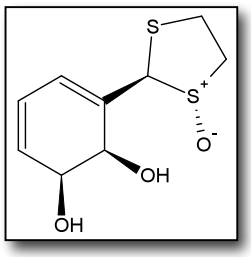
135 (2.49g, 15%), colourless crystals; mp 54-57 ^oC (from Et_2O/hexane) (5%MeOH/$CHCl_3$); $[\alpha]_D$ + 32.8 (*c* 0.6, $CHCl_3$); (Found: C, 50.2; H, 5.7, $C_9H_{12}O_2S_2$ requires C, 50.0; H, 5.6 %); δ_H (500 MHz, $CDCl_3$) 3.26-3.37 (4 H, m, CH_2), 4.34 (1 H, d, $J_{1,2}$ 5.7, 2-H), 4.44 (1 H, dd, $J_{2,1}$ 5.7, 3.0, 1-H), 5.37 (1 H, s, 2'-H), 5.85-5.92 (2

H, m, , 5- & 6-H), 6.05 (1 H, d, *J* 5.3, 4-H); δ_C (125 MHz, $CDCl_3$) 39.61, 39.78, 55.65, 67.27, 70.19, 122.55, 123.11, 132.01, 138.03; m/z (EI) 216 M^+, 80%), 198 (M^+-H_2O, 100%); CD: λ 264nm $\Delta\varepsilon$ 2.01, λ 215nm $\Delta\varepsilon$ - 1.25.



(-)-*trans*-(*1S,2S*)-2-Phenyl-1,3-dithiolane-1-oxide 134.

134 (0.46g, 3%) colourless crystalline solid; mp 85-89 °C (from MeOH) (lit.[144] 66-67 °C, racemic); $[\alpha]_D$ - 102.8 (*c* 0.8, $CHCl_3$) (- 128.9 enantiopure); δ_H (500 MHz, $CDCl_3$) 2.88-2.93 (1 H, m, 5-C$H_A$$H_B$), 3.32-3.36 (1 H, m, 4-C$H_A$$H_B$), 3.62 (1 H, m, 4-C$H_A$$H_B$), 3.80-3.85 (1 H, m, 5-C$H_A$$H_B$), 5.40 (1 H, s, 2-H), 7.33-7.39 (3 H, m, Ar), 7.48 (2 H, d, *J* 6.9, Ar); δ_C (125 MHz, $CDCl_3$) 32.44, 53.27, 77.99, 128.51, 129.01, 133.14; m/z (EI) 198 (M^+, 100%), 121 (M^+-C_6H_5, 99%), 77 (M^+-$C_3H_5OS_2$, 82%); CD: λ 228nm $\Delta\varepsilon$ - 9.43.



(+)-*cis*-(*1S,2R*)-3-(*trans*-(*1'S,2'S*)-1',3'-Dithiolan-2'-yl-1'-oxy)cyclohexa-3,5-diene-1,2-diol 136.

136 (1.02g, 8%), unstable colourless oil; $[\alpha]_D$ + 62.0 (*c* 1.6, $CHCl_3$); (Found: M^+, 232.0232. $C_9H_{12}O_3S_2$ requires 232.0228); δ_H (500 MHz, $CDCl_3$) 3.12-3.17 (1 H, m, 4'-C$H_A$$H_B$), 3.39-3.44 (1 H, m, 5'-C$H_A$$H_B$), 3.54-3.59 (1 H, m, 4'-C$H_A$$H_B$), 3.84-3.90 (1 H, m, 5'-C$H_A$$H_B$), 4.44 (1 H, d, $J_{1,2}$ 6.0, 2-H), 4.47 (1 H, m, 1-H), 5.17 (1 H, s, 2'-H), 5.94-6.02 (2 H, m, 5- & 6-H), 6.25 (1 H, d, *J* 5.3, 4-H); δ_C (125 MHz, $CDCl_3$) 32.35, 56.74, 65.69, 69.64, 72.84, 122.53, 128.66, 130.70, 133.95; m/z (EI) 232 (M^+, 15%), 214 (M^+-H_2O, 35%); CD: λ 264nm $\Delta\varepsilon$ 5.33, λ 218nm $\Delta\varepsilon$ - 1.66.

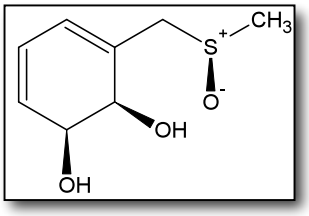
5.12. Sulfoxidation of *cis*-dihydrodiols 85, 123, 124 and 129 using dimethyldioxirane.

Dimethyldioxirane (DMD) was prepared as a solution in acetone by addition of potassium peroxymonosulfate (oxone) to a mixture of water, acetone and sodium hydrogen carbonate in accordance with the literature procedure.[144] A solution of DMD (0.08M) was added, dropwise, to a stirring solution of the diol in acetone maintained at

0 ^{o}C. The progress of the reaction was constantly monitored by TLC (8% MeOH/$CHCl_3$)

When all of the diol had oxidised to the diolsulfoxides, the reaction was terminated by the removal of the solvent *in vacuo* to yield a mixture of diastereoisomers:

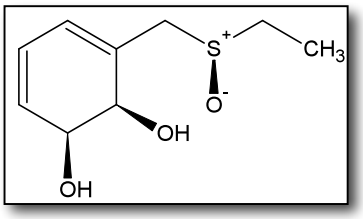
The crude mixture, obtained from DMD oxidation of diol **85** (2g, 11.6mmol), was separated by column chromatography (8% MeOH/$CHCl_3$) into two diolsulfoxides **125** and **122**.



(+)-*cis*-(1*S*,2*R*)-3-(*R*-Methylsulfinylmethyl]-3,5-cyclohexadiene-1,2-diol 125.

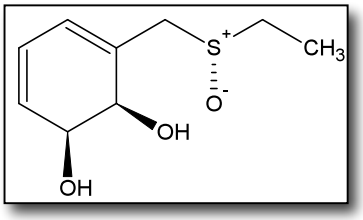
Diolsulfoxide **125** (0.98g, 45%), colourless crystalline solid; mp 123 ^{o}C (from MeOH/$CHCl_3$); R_f 0.28 (10% MeOH/$CHCl_3$); $[\alpha]_D$ + 56.2 (*c* 2.2, MeOH); (Found: C, 50.9. H, 6.5; $C_8H_{12}O_3S$ requires C, 51.1. H, 6.4%); (Found: M^+, 188.0511. $C_8H_{12}O_3S$ requires 188.0507); (Found: M^+-H_2O, 170.0402. $C_8H_{10}O_2S$ requires 170.0406); δ_H (500 MHz, $CDCl_3$) 2.59 (3 H, s, Me), 3.25 (1 H, d, $J_{A,B}$ 13.3, CH_AH$_B$), 4.08 (1 H, d, $J_{B,A}$ 13.3, CH$_A$$H_B$), 4.18 (1 H, d, $J_{1,2}$ 6.0, 2-H), 4.33 (1 H, m, 1-H), 5.94 (1 H, d, *J* 5.2, 4-H), 6.02 (1 H, m, 5-H), 6.07 (1 H, m, 6-H); δ_C (125 MHz, $CDCl_3$) 34.14, 54.78, 66.22, 67.81, 121.72, 126.20, 128.92, 129.81; m/z (EI) 170 (M^+-H_2O, 7%); CD: λ 303nm $\Delta\varepsilon$ - 0.49, λ 267nm $\Delta\varepsilon$ 1.49, λ 225nm $\Delta\varepsilon$ 0.80. Diolsulfoxide **122** (0.98g, 45%); R_f 0.23, was found to have identical physical and spectral characteristics to the sample isolated from biotransformation of benzylmethyl sulfide **81**.

Diol **123** (5g, 26.9mmol), on oxidation with DMD, gave a mixture of two products; PLC (8% MeOH/$CHCl_3$) separation yielded two diolsulfoxides **140** and **141**:



(+)-*cis*-(1*S*,2*R*)-3-(*R*-Ethylsulfinylethyl)-3,5-cyclohexadiene-1,2-diol 140.

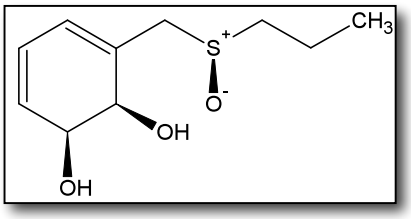
Diolsulfoxide **140** (2.45g, 45%),viscous colourless oil; R_f 0.40 (8% MeOH/$CHCl_3$); $[\alpha]_D$ + 53.1 (*c* 0.6, MeOH); (required, M^+: 202.0664, $C_9H_{14}O_3S$: 202.0665); (required, M^+-H_2O: 184.0562, $C_9H_{12}O_2S$: 184.0558); δ_H (500 MHz, $CDCl_3$) 1.30 (3 H, t, *J* 7.5, Me), 2.70 (1 H, m, C$H_A$$H_B$Me), 2.82 (1 H, m, C$H_A$$H_B$Me), 3.39 (1 H, d, $J_{A,B}$ 13.2, C$H_A$$H_B$SO), 3.89 (1 H, d, $J_{B,A}$ 13.2, C$H_A$$H_B$SO), 4.15 (1 H, d, $J_{1,2}$ 6.0, 2-H), 4.26 (1 H, m, 1-H), 5.94-6.05 (3 H, m, 4-,5- & 6-H); δ_C (125 MHz, $CDCl_3$) 7.11, 43.90, 54.68, 67.66, 69.67, 123.74, 127.09, 130.98, 131.20; m/z (EI) 184 (M^+-H_2O, 2%), 125 (M^+-C_2H_5OS, 70%), 107 (M^+-$C_2H_7O_2S$); CD: λ 269nm $\Delta\varepsilon$ 1.12, λ 242nm $\Delta\varepsilon$ - 0.30, λ 224nm $\Delta\varepsilon$ 0.59.



(+)-*cis*-(1*S*,2*R*)-3-(*S*-Ethylsulfinylethyl)-3,5-cyclohexadiene-1,2-diol 141.

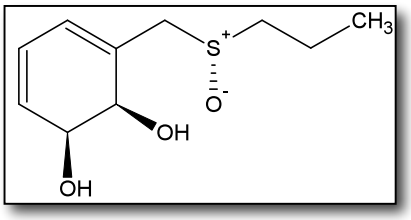
Diolsufoxide **141** (2.45g, 45%), viscous colourless oil; R_f 0.34 (8% MeOH/$CHCl_3$); $[\alpha]_D$ + 187 (*c* 0.7, MeOH); (required, M^+: 202.0664, $C_9H_{14}O_3S$: 202.0665); δ_H (500 MHz, $CDCl_3$) 1.34 (3 H, t, *J* 7.5, Me), 2.72-2.78 (2 H, m, CH_2Me), 3.58 (1 H, d, $J_{A,B}$ 12.9, C$H_A$$H_B$SO), 3.80 (1 H, d, $J_{B,A}$ 12.9, C$H_A$$H_B$SO), 4.26 (1 H, m, 1-H), 4.30 (1 H, d, $J_{1,2}$ 6.1, 2-H), 6.01-6.09 (3 H, m, 4-,5- & 6-H); δ_C (125 MHz, $CDCl_3$) 6.93, 44.96, 54.57, 66.69, 70.11, 124.73, 125.99, 129.69, 132.57; m/z (EI) 184 (M^+-H_2O, 2%), 125 (M^+-C_2H_5OS, 70%), 107 (M^+-$C_2H_7O_2S$); CD: λ 270nm $\Delta\varepsilon$ 6.03, λ 234nm $\Delta\varepsilon$ - 1.13, λ 205nm $\Delta\varepsilon$ -2.24.

Diol **124** (1g, 5.0mmol) was oxidised (DMD) and the product mixture separated by PLC (8% MeOH/$CHCl_3$) into diolsulfoxides **142** and **143**:



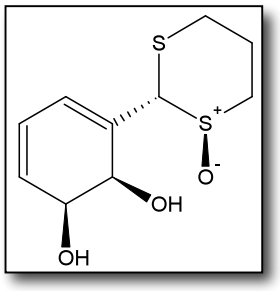
(+)-*cis*-(1*S*,2*R*)-3-(*R*-Propylsulfinylmethyl)cyclohexa-3,5-diene-1,2-diol a 142.

Diolsulfoxide **142** (0.49g, 45%), viscous oil; R_f 0.24 (8% MeOH/$CHCl_3$); $[\alpha]_D$ + 77.6 (*c* 3.0, $CHCl_3$); (Found: M^+, 216.0818. $C_{10}H_{16}O_3S$ requires 216.0820); (Found: M^+-H_2O, 198.0718. $C_{10}H_{14}O_2S$ requires 198.0715); δ_H (500 MHz, $CDCl_3$) 1.11 (3 H, t, *J* 7.2, Me), 1.75-1.83 (2 H, m, C*H*$_2$Me), 2.59 (1 H, m, CH_AH$_B$Et), 2.90 (1 H, m, CH$_A$$H_B$Et), 3.32 (1 H, d, $J_{A,B}$ 13.3, CH_AH$_B$SOPr), 4.02 (1 H, d, $J_{B,A}$ 13.3, CH$_A$$H_B$SOPr), 4.19 (1 H, d, $J_{2,1}$ 5.8, 2-H), 4.33 (1 H, m, 1-H), 5.93 (1 H, d, *J* 5.1, 4-H), 6.00 (1 H, dd, *J* 5.2, 9.7, 5-H), 6.06 (1 H, dd, *J* 3.5, 9.6, 6-H); δ_C (125 MHz, $CDCl_3$) 13.42, 16.45, 52.02, 54.98, 67.85, 69.58, 123.58, 127.40, 131.19, 131.34; m/z (EI) 198 (M^+-H_2O, 2.5%), 125 (M^+-C_3H_7OS, 48%), 107 (M^+-$C_3H_9O_2S$, 68%); CD: λ 268nm $\Delta\varepsilon$ 0.94, λ 229nm $\Delta\varepsilon$ 0.33, λ 223nm $\Delta\varepsilon$ 0.21, and compound **143**.



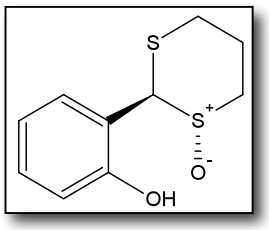
(+)-*cis*-(*R*)-(1*S*,2*R*)-3-(*S*-Propylsulfinylmethyl) cyclohexa-3,5-diene-1,2-diol 143.

Diolsulfoxide **143** (0.49g, 45%), a viscous oil; R_f 0.19 (10% MeOH/$CHCl_3$); $[\alpha]_D$ + 60.3 (*c* 1.5, $CHCl_3$); (Found: M^+-H_2O, 198.0714. $C_{10}H_{16}O_3S$ requires 198.0715); δ_H (500 MHz, $CDCl_3$) 1.10 (3 H, t, *J* 7.4, Me), 1.77-1.84 (2 H, m, C*H*$_2$Me), 2.63 (1 H, m, CH_AH$_B$Et), 2.76 (1 H, m, CH$_A$$H_B$Et), 3.57 (1 H, d, $J_{A,B}$ 13.0, CH_AH$_B$SOPr), 3.82 (1 H, d, $J_{B,A}$ 13.0, CH$_A$$H_B$SOPr), 4.26 (1 H, dd, $J_{1,6}$ 3.8, $J_{1,2}$ 5.8, 1-H), 4.30 (1 H, d, $J_{2,1}$ 5.6, 2-H), 6.01 (1 H, m, *J* 4.9, 5-H), 6.07 (1 H, d, *J* 4.7, 4-H), 6.08 (1 H, m, 6-H); δ_C (125 MHz, $CDCl_3$) 13.42, 16.41, 53.42, 54.99, 66.63, 70.19, 124.82, 125.96, 129.66, 132.54; m/z (EI) 198 (M^+-H_2O, 4%), 125 (M^+-C_3H_7OS, 55%), 107 (M^+-$C_3H_9O_2S$, 100%); CD: λ 270nm $\Delta\varepsilon$ 4.47, λ 238nm $\Delta\varepsilon$ - 2.98, λ 220nm $\Delta\varepsilon$ 0.66.



(-)-*cis*-(*1S,2R*)-3-(*trans-1'R,2'R*-1',3'-Dithan-2'-yl-1'-oxy) cyclohexa-3,5-diene-1,2-diol 144.

Dithiane diol **129** (200mg, 0.87mmol) was oxidised (DMD) and the product mixture separated by PLC (5% MeOH/$CHCl_3$) into two dithiane diolsulfoxides **144** and **130**. Diolsulfoxide **144** (96mg, 45%), viscous oil; R_f 0.14 (5% MeOH/$CHCl_3$); $[\alpha]_D$ - 47.8 (*c* 1.5, $CHCl_3$); (Found: M^+, 246.0308. $C_{10}H_{14}O_3S_2$ requires 246.0384); δ_H (500 MHz, $CDCl_3$) 2.23-2.38 (1 H, m, $R_2CH_AH_B$), 2.47-2.51 (1 H, m, $R_2CH_AH_B$), 2.65-2.70 (1 H, m, SCH_AH_B), 2.75-2.85 (2 H, m, SCH_AH_B & $SOCH_AH_B$), 3.49-3.53 (1 H, m, $SOCH_AH_B$), 4.31 (1 H, dd, $J_{1,6}$ 3.5, $J_{1,2}$ 6.1, 1-H), 4.39 (1 H, d, $J_{2,1}$ 6.1, 2-H), 4.61 (1 H, s, SCH), 6.03-6.08 (3 H, m, 4-,5- & 6-H); δ_C (125 MHz, $CDCl_3$) 29.61, 30.84, 54.93, 66.53, 67.12, 69.84, 124.39, 125.08, 130.34, 134.57; m/z (EI) 246 (M^+, 90%), 228 (M^+-H_2O, 50%); CD: λ 270nm $\Delta\varepsilon$ - 1.14, λ 230nm $\Delta\varepsilon$ 4.06. Diolsulfoxide **130** (95mg, 45%); R_f 0.17; colourless crystals; mp 155-157 ^oC ($CHCl_3$), was found to have identical physical and spectral characteristic to the sample isolated as a metabolite of 2-phenyl-1,3-dithiane **110**.



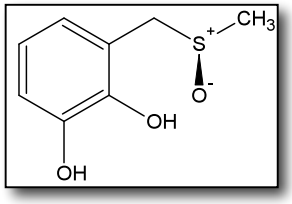
***trans*-(2-Hydroxyphenyl)-1,3-dithiolane-1-oxide 145.**

A solution of diolsulfoxide **130** (100mg, 0.41mmol), in $CHCl_3$ (5 cm^3), was heated at 70 ^oC in the presence of *p*-toluene sulfonic acid (5mg). The progress of reaction was followed by TLC. When all the diol had aromatised, the crude reaction mixture was crystallised to yield phenol sulfoxide **145** (25mg, 27%), a white crystalline solid; mp 95 ^oC (from $CHCl_3$); R_f 0.49 (7% MeOH/$CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.29-2.39 (1 H, m, $R_2CH_AH_B$), 2.53-2.58 (1 H, m, $R_2CH_AH_B$), 2.73-2.78 (1 H, m, SCH_AH_B), 2.88-2.96 (2 H, m, SCH_AH_B & $SOCH_AH_B$), 3.61-3.67 (1 H, m, $SOCH_AH_B$), 5.36 (1 H, s, CH), 6.85 (1 H, d, *J* 8.1, Ar), 6.94 (1 H, m, Ar), 7.14 (1 H, m, Ar), 7.20 (1 H, d, *J* 7.7, Ar), 8.55 (1 H, s, OH); δ_C (125 MHz, $CDCl_3$) 29.51,

31.08, 54.93, 62.99, 118.52, 120.99, 121.12, 127.55, 130.55, 156.06; m/z (EI) 228 (M^+, 80%).

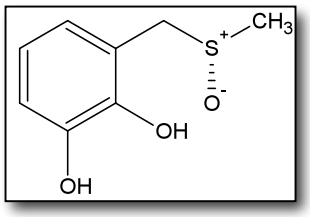
5.13. Biotransformation of diol sulfoxides with *E. coli* (narB).

The biotransformation media, from the biotransformation of diol sulfoxide (*e.g,* **122**, **125**, **140**, **141**, **142** and **143)** was evaporated and the viscous concentrate repeatedly extracted with EtOAc. The crude evaporated extract-residue was purified by PLC:



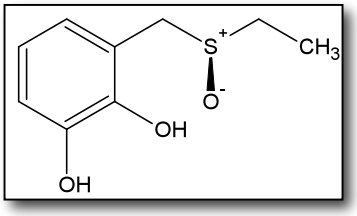
(-)-3-(*R*-Methylsulfinylmethyl)catechol (*R*)-146.

Enzyme catalysed dehydogenation of diol sulfoxide **125** (200mg, 1.1mmol) using the *E. coli* (nar B) strain yielded the crude catechol **146** which was purified by PLC (10% MeOH/$CHCl_3$) giving catachol sulfoxide **146** (137mg, 69%) as a light yellow coloured semi-solid; R_f 0.28 (10% MeOH/$CHCl_3$); $[\alpha]_D$ – 70.9 (*c* 2.2, $CHCl_3$); (required, M^+: 186.0351, $C_8H_{10}O_3S$: 186.0351); δ_H (500 MHz, $CDCl_3$) 2.49 (3 H, s, Me), 3.80 (1 H, d, $J_{A,B}$ 14.2, CH_AH$_B$), 4.44 (1 H, d, $J_{B,A}$ 14.2, CH$_A$$H_B$), 6.56 (1 H, d, *J* 7.7, Ar), 6.63 (1 H, s, OH), 6.78 (1 H, m, Ar), 6.89 (1 H, d, *J* 8.0, Ar), 9.10 (1 H, s, OH); δ_C (125 MHz, $CDCl_3$) 35.66, 54.30, 115.93, 117.53, 121.07, 122.89, 143.59, 147.03; m/z (EI) 186 (M^+, 17%), 123 (M^+-CH_3SO, 100%).



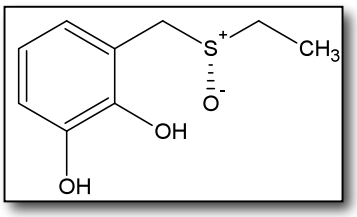
(+)-3-(*S*-Methylsulfinylmethyl)catechol (*S*)-146.

The title catechol sulfoxide enantiomer **(*S*)-146** (24mg, 24%), semi-solid, $[\alpha]_D$ + 69.5 (*c* 1.4, $CHCl_3$), was similarly obtained by the enzyme catalysed dehydogenation of diol sulfoxide **122** (100mg, 0.55mmol) with the *E. coli* (nar B) strain.



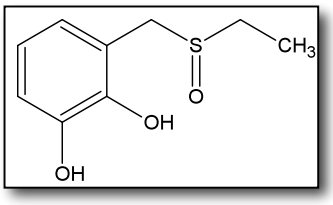
(-)-3-(*R*-Ethylsulfinylmethyl) catechol (*R*)-147.

Diol Sulfoxide substrate **140** (100mg, 0.50mmol), on dehydrogenation with *E. coli* (nar B) strain yielded crude catechol **147**. Purification by PLC (10% MeOH/$CHCl_3$) gave catechol sulfoxide **147** (20mg, 20%), a semi-solid solid; R_f 0.30 (10% MeOH/$CHCl_3$); $[\alpha]_D$ – 52.2 (*c* 1.3, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.30 (3 H, m, Me) 2.59-2.66 (1 H, m, C$H_A$$H_B$Me), 2.70-2.76 (1 H, m, C$H_A$$H_B$Me), 3.81 (1 H, d, *J* 14.4, ArC$H_A$$H_B$), 4.46 (1 H, d, *J* 14.4, ArC$H_A$$H_B$), 6.00 (1 H, s, OH), 6.54 (1 H, d, *J* 7.6, Ar), 6.80 (1 H, m, Ar), 6.92 (1 H, d, *J* 8.0, Ar), 9.78 (1 H, s, OH); δ_C (125 MHz, $CDCl_3$) 6.98, 43.47, 52.38, 155.60, 118.08, 121.18, 122.56, 143.65, 147.57; m/z (EI) 200 (M^+, 20%), 123 (M^+-C_2H_5SO, 100%).



(+)-3-(*S*-Ethylsulfinylmethyl)catechol (*S*)-147.

The title catechol sulfoxide enantiomer **(*S*)-147** (19mg, 19%), a semi-solid, $[\alpha]_D$ + 53.5 (*c* 1.0, $CHCl_3$) was similarly obtained from the *E. coli* (nar B) metabolism of diol **141** (100mg, 0.55mmol).

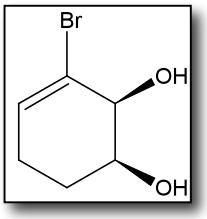


3-(Propylsulfinyl)methyl catechol 148.

Enzyme catalysed dehydogenation (nar B) of a mixture of diol sulfoxides **142** and **143** (90mg, 0.42mmol), gave a racemic sample of crude catechol **148**. Purification by PLC (10% MeOH/$CHCl_3$) furnished a pure racemic sample of catechol sulfoxide **148** (11mg, 12%), semi-solid solid; R_f 0.33 (10% MeOH/$CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.25 (3 H, t, *J* 7.0, Me), 1.73-1.79 (2 H, m, CH_2Me), 2.44-2.49 (1 H, m, SC$H_A$$H_B$Et),

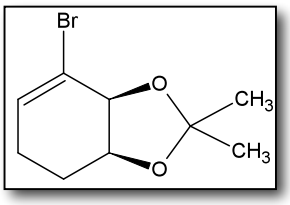
2.51-56 (1 H, m, SCH_AH_BEt), 3.78 (1 H, d, *J* 14.4, $ArCH_AH_B$), 4.48 (1 H, d, *J* 14.4, $ArCH_AH_B$), 6.00 (1 H, s, OH), 6.54 (1 H, d, *J* 7.6, Ar), 6.80 (1 H, m, Ar), 6.91 (1 H, d, *J* 8.0, Ar), 9.80 (1 H, s, OH); δ_C (125 MHz, $CDCl_3$) 13.37, 16.30, 52.88, 53.58, 115.58, 118.20, 121.18, 122.59, 143.63, 147.58; m/z (EI) 214 (M^+, 29%), 123 (M^+-C_3H_7SO, 100%).

5.14. Chemoenzymatic synthesis of diol sulfoxides using bromobenzene *cis*-dihydrodiol 34.



(-)-*cis*-(1*S*,2*S*)-1,2-Dihydroxy-3-bromocyclohex-3-ene 152.

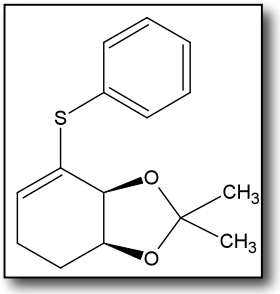
A solution of *cis*-(1*S*,2*S*)-1,2-dihydroxy-3-bromocyclohexa-3,5-diene **34** (1g, 5.24mmol) in THF (15 cm^3) was hydrogenated (5hr at 25psi) in the presence of rhodium on alumina (500mg, 5%). The catalyst was filtered off, the solvent evaporated and the crude hydrogenated product purified by column chromatography (5% MeOH/$CHCl_3$) to yield tetrahydrodiol **152** (860mg, 85%) as a white silky needles; mp 104-107°C (from CH_2Cl_2) (lit.[145] 105-107°C); $[\alpha]_D$ – 112 (*c* 1.2, MeOH) (lit.[145] – 114, MeOH); δ_H (500 MHz, $CDCl_3$) 1.74-1.88 (2 H, m, 6-H), 2.05-2.14 (1 H, m, 5-CH_AH_B), 2.24-3.32 (1 H, m, 5-CH_AH_B), 2.59 (1 H, d, *J* 5.1, OH), 2.68 (1 H, d, *J* 3.6, OH), 3.95 (1 H, m, 1-H), 4.21 (1 H, d, *J* 3.9, 2-H), 6.23 (1 H, m, 4-H); δ_C (125 MHz, $CDCl_3$) 25.13, 25.18, 69.03, 71.80, 122.39, 132.81; m/z (EI) 194 (M^+(^{81}Br), 3%), 192 (M^+(^{79}Br), 3%).



(+)-*cis*-(3a*S*,7a*S*)-7-Bromo-2,2-dimethyl-3a,4,5,7a-tetrahydro-1,3-benzodioxole 154.

A suspension of tetrahydrodiol **152** (1g, 5.18mmol), in 2,2-dimethoxypropane (5cm^3) was stirred overnight at room temperature in the presence of *p*-toluenesulfonic acid (2mg); the solvent was evaporated off, the residue taken up in Et_2O solvent (75 cm^3),

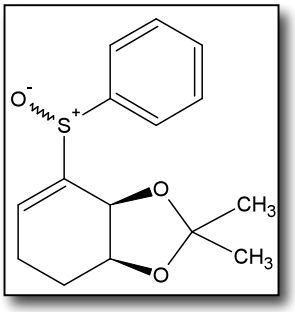
washed with water and dried (Na_2SO_4). The crude product, obtained after removal of Et_2O, was purified by column chromatography (25%Et_2O/hexane) to yield acetonide **154** (785mg, 65%) as a crystalline solid; mp 41 ^oC (from Et_2O/hexane); $[\alpha]_D$ + 80.7 (*c* 0.8, $CHCl_3$); (Found: M^+-CH_3, 216.9864. $C_8H_{10}O_2{}^{79}Br$ requires 216.9867); δ_H (500 MHz, $CDCl_3$) 1.41 (3 H, s, C*Me*$_A$Me$_B$), 1.45 (3 H, s, CMe$_A$*Me*$_B$), 1.80-1.83 (1 H, m, 5-C*H*$_A$H$_B$), 2.03-2.07 (2 H, m, 5-CH$_A$*H*$_B$ & 6-C*H*$_A$H$_B$), 2.27-2.33 (1 H, m, 6-CH$_A$*H*$_B$), 4.41 (1 H, m, 1-H), 4.50 (1 H, d, *J* 5.3, 2-H), 6.23 (1 H, dd, *J* 5.7, 2.5, 4-H); δ_C (125 MHz, $CDCl_3$) 21.45, 23.41, 25.51, 26.64, 73.26, 75.56, 108.31, 121.34, 131.21; m/z (EI) 219 (M^+(^{81}Br)-CH_3, 33%), 217 (M^+(^{79}Br)-CH_3, 33%).



(-)-*cis*-(3a*S*,7a*S*)-7-Phenylsulfanyl-2,2-dimethyl-3a,4,5,7a-tetrahydro-1,3-benzodioxole 155.

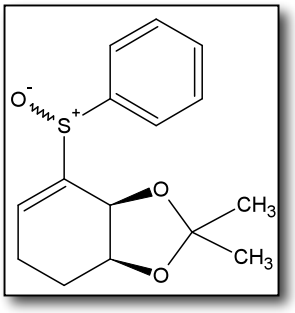
A solution of acetonide **154** (4g, 17.2mmol) in dry THF (100 cm^3) was treated with a solution of t-butyllithium in hexane (11.5 cm^3, 17.2mmol, 1.5M) at – 80 ^oC under a N_2 gas atmosphere. After stirring the reaction mixture for 1hr, a solution of diphenyldisulfide (4.9g, 22.4mmol) in THF (15 cm^3) was added to it dropwise; it was left for stirring at – 80 ^oC for another hour and then at – 41 ^oC overnight. The solvent was evaporated off, the crude product was taken up in Et_2O (150 cm^3) and the Et_2O solution washed with (5%) aqueous K_2CO_3 solution. The crude mixture, left behind after removal of Et_2O, was purified by column chromatography (Et_2O/hexane) to give acetonide sulfide **155** (4.20g, 93%) as a yellow oil, which crystallised on standing; mp 52 ^oC (from MeOH); R_f 0.45 (25% Et_2O/hexane); $[\alpha]_D$ – 28 (*c* 1.5, $CHCl_3$); (Found: M^+, 262.1015. $C_{15}H_{18}O_2S$ requires 262.1028); δ_H (500 MHz, $CDCl_3$) 1.36 (3 H, s, C*Me*$_A$Me$_B$), 1.44 (3 H, s, CMe$_A$*Me*$_B$), 1.78 (1 H, m, 5-C*H*$_A$H$_B$), 1.99 (1 H, m, 5-CH$_A$*H*$_B$), 2.07 (1 H, m, 6-C*H*$_A$H$_B$), 2.32 (1 H, m, 6-CH$_A$*H*$_B$), 4.38 (2 H, m, 1- & 2-H), 6.10 (1 H, dd, *J* 5.3, *J* 3.1, 4-H), 7.23-7.31 (3 H, m, Ar), 7.40 (2 H, d, *J* 7.4, Ar); δ_C (125 MHz, $CDCl_3$) 21.95, 25.33, 26.33, 27.75, 73.26, 73.69, 108.92, 127.02, 129.02, 131.30, 132.34, 134.02, 134.99; m/z (EI) 262 (M^+, 68%), 247 (M^+-CH_3, 13%).

To a solution of acetonide sulfide **155** (1g, 3.8mmol) in $CHCl_3$ (50 cm^3), maintained at 0 ^oC was added dropwise (30 min.) a solution of *M*CPBA (0.7g, 4.1mmol) in $CHCl_3$ (50 cm^3); the mixture was stirred for 3hr. The organic layer was separated, washed with aqueous (5%) K_2CO_3 solution, dried (Na_2SO_4) and the solvent evaporated off. The crude product was separated by PLC (Et_2O) into two acetonide sulfoxide **156, 157** and sulfone **158**:



(-)-*cis*-(3a*S*,7a*S*)-2,2-Dimethyl-7-(phenylsulfinyl)-3a,4,5,7atetrahydro-1,3-benzodioxole 156.

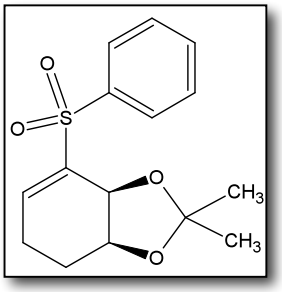
Sulfoxide **156** (0.48g, 45%), a white crystalline solid; mp 87-90 ^oC (from Et_2O); R_f 0.50 (Et_2O); $[\alpha]_D$ – 48.6 (*c* 1.5, $CHCl_3$); (Found: M^+, 278.0982. $C_{15}H_{18}O_3S$ requires 278.0977); (Found: C, 64.3; H, 6.6. $C_{15}H_{18}O_3S$ requires C, 64.7; H, 6.52 %); δ_H (500 MHz, $CDCl_3$) 1.32 (6 H, s, Me), 1.64 (1 H, m, 6-CH_AH$_B$), 2.06 (1 H, m, 6-CH$_A$$H_B$), 2.23 (1 H, m, 5-C$H_AH_B$), 2.45 (1 H, m, 5-CH$_A$$H_B$), 4.17 (1 H, d, *J* 5.4, 2-H), 4.32 (1 H, m, 1-H), 6.78 (1 H, dd, *J* 5.5, *J* 2.7, 4-H), 7.49 (3 H, m, Ar), 7.69 (2 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 20.58, 24.31, 26.52, 27.57, 69.33, 73.34, 109.90, 125.47, 127.55, 130.73, 131.31, 142.81, 142.82; m/z (EI) 278 (M^+, 15%), 263 (M^+-CH_3, 11%), 220 (M^+-C_3H_6O, 100%).



(+)-*cis*-(3a*S*,7a*S*)-2,2Dimethyl-7-(phenylsulfinyl)-3a,4,5,7a-tetrahydro-1,3-benzodioxole 157.

Sulfoxide **157** (0.48g, 45%), a colourless crystalline solid; mp 80-82^oC (from Et_2O); R_f 0.36 (Et_2O); $[\alpha]_D$ + 121 (*c* 1.5, $CHCl_3$); (Found: M^+, 278.0982. $C_{15}H_{18}O_3S$ requires 278.0977); (Found: C, 64.3; H, 6.6. $C_{15}H_{18}O_3S$ requires C, 64.7; H, 6.5 %); δ_H (500 MHz, $CDCl_3$) 1.05 (3 H, s, CMe_AMe$_B$), 1.27 (3 H, s, CMe$_A$$Me_B$), 1.67 (1 H, m, 6-

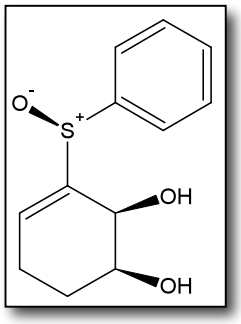
CH_AH$_B$), 2.02 (1 H, m, 6-CH$_A$$H_B$), 2.20 (1 H, m, 5-C$H_AH_B$), 2.40 (1 H, m, 5-CH$_A$$H_B$), 4.38 (1 H, m, 1-H), 4.68 (1 H, d, *J* 5.8, 2-H), 6.74 (1 H, dd, *J* 5.9, *J* 2.8, 4-H), 7.47 (3 H, m, Ar), 7.68 (2 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 20.84, 25.41, 26.09, 26.99, 70.22, 73.47, 109.40, 125.60, 128.88, 130.97, 135.18, 143.48, 143.73; m/z (EI) 278 (M^+, 15%), 263 (M^+-CH_3, 11%), 220 (M^+-C_3H_6O, 100%).



(-)-*cis*-(3a*S*,7a*S*)-2,2-Dimethyl-7-(phenylsulfonyl)-3a,4,5,7a-tetrahydro-1,3-benzodioxole 158.

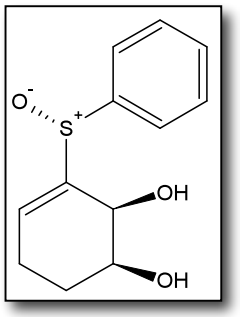
Sulfone **158** was a minor product (56mg, 5%), white crystalline solid; mp 129^{o}C (from MeOH); R_f 0.76 (Et_2O); $[\alpha]_D$ - 154 (*c* 1, $CHCl_3$) (Found: M^+, 294.0926. $C_{15}H_{18}O_4S$ requires 294.0941); (Found: C, 61.1; H, 6.3. $C_{15}H_{18}O_4S$ requires C, 61.2; H, 6.2 %); δ_H (500 MHz, $CDCl_3$) 0.69 (3 H, s, CMe_AMe$_B$), 1.23 (3 H, s, CMe$_A$$Me_B$), 1.71 (1 H, m, 6-C$H_AH_B$), 2.07 (1 H, m, 6-CH$_A$$H_B$), 2.27 (1 H, m, 5-C$H_AH_B$), 2.41 (1 H, m, 5-CH$_A$$H_B$), 4.42 (1 H, m, 1-H), 4.98 (1 H, d, *J* 4.7, 2-H), 7.24 (1 H, dd, *J* 6.0, *J* 2.4, 4-H), 7.48 (2 H, m, Ar), 7.57 (1 H, m, Ar), 7.92 (2 H, d, *J* 7.3, Ar); δ_H (125 MHz, $CDCl_3$) 20.76, 24.21, 26.31, 26.49, 70.26, 72.94, 109.07, 128.57, 128.75, 132.99, 140.51, 141.35, 142.42; m/z (EI) 295 ($M^{+\bullet}$+1, 3%), 279 ($M^{+\bullet}$-CH_3, 100%).

A solution of unseparated acetonide sulfoxides **156** and **157** (1:1, 400mg, 1.45mmol) in TFA/H_2O/THF (1:2:8, 30 cm^3) was kept at 40 ^{o}C for 8hr. On completion of the deprotection reaction, monitored by TLC, the solvent mixture was evaporated under reduced pressure and the residue taken up in EtOAc (50 cm^3); the EtOAc extract was washed with a saturated solution of $NaHCO_3$, dried (Na_2SO_4) and the solvent removed. The crude mixture was separated by PLC into diolsulfoxides **160** and **159**:



(-)-*cis*-(1*S*,2*S*)-3-(*S*-Phenylsulfinyl)cyclohex-3-ene-1,2-diol 160.

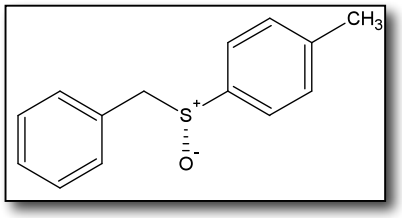
Diolsulfoxide **160** (120mg, 35%), white crystalline solid; mp 145-150 °C (from Et_2O); R_f 0.35 (8% MeOH/$CHCl_3$); $[\alpha]_D$ − 6.6 (*c* 1, $CHCl_3$); (Found: M^+, 238.0658. $C_{12}H_{14}O_3S$ requires 238.0664); δ_H (500 MHz, $CDCl_3$) 1.58 (1 H, m, 6-CH_AH$_B$), 1.95 (1 H, m, 6-CH$_A$$H_B$), 2.19 (1 H, m, 5-C$H_AH_B$), 2.58 (1 H, m, 5-CH$_A$$H_B$), 3.65 (1 H, m, 1-H), 4.29 (1 H, d, *J* 4.0, 2-H), 6.77 (1 H, dd, *J* 7.5, *J* 3.8, 4-H), 7.51-7.63 (5 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 23.80, 25.05, 66.21, 67.87, 124.39, 129.38, 131.06, 139.03, 141.15, 141.48; m/z (EI) 238 (M^+, 15%), 220 (M^+-H_2O, 5%).



(-)-*cis*-(1*S*,2*S*)-3-(*R*-Phenylsulfinyl)cyclohex-3-ene-1,2-diol 159.

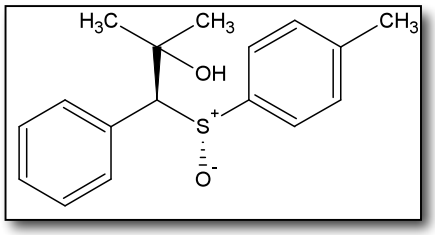
Diolsulfoxide **159** (120mg, 35%), a semi-solid; R_f 0.32 (8% MeOH/$CHCl_3$); $[\alpha]_D$ − 88.5 (*c* 1.0, $CHCl_3$); (Found: M^+, 238.0658. $C_{12}H_{14}O_3S$ requires 238.0664); δ_H (500 MHz, $(CD_3)_2CO$) 1.43-1.49 (1 H, m, 6-CH_AH$_B$), 1.66-1.75 (1 H, m, 6-CH$_A$$H_B$), 2.03-2.13 (1 H, m, 5-CH_AH$_B$), 2.28-2.37 (1 H, m, 5-CH$_A$$H_B$), 3.54 (1 H, m, 1-H), 3.71 (2 H, m, 2-H & OH), 3.88 (1 H, m, OH), 6.47 (1 H, m, 4-H), 7.41 (3 H, m, Ar), 7.57 (2 H, m, Ar); δ_C (125 MHz, $(CD_3)_2CO$) 23.00, 25.39, 64.64, 68.24, 125.10, 128.95, 129.79, 130.76, 144.39, 144.58; m/z (EI)) 238 (M^+, 13%), 220 (M^+-H_2O, 15%).

5.15. Synthesis of enantiopure benzyl-*para*-tolyl sulfoxide 91.



(+)-(*R*)-Benzyl-*p*-tolylsulfoxide (*R*)-91.

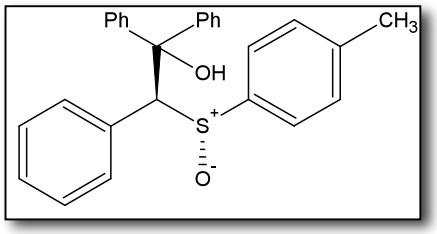
Benzyl magnesium bromide solution (4.1cm^3, 4.1mmol, 1M, Et_2O) was added (30min), at 0 ^oC, to a solution of (*R*)-menthyl-*p*-tolyl sulfinate **(*R*)-163** (1g, 3.4mmol) in dry THF (30 cm^3). After stirring (1hr) the reaction mixture at room temperature the solvent was evaporated and EtOAc (75 cm^3) was added to the residue. The mixture was shaken with a solution HCl (50 cm^3, 3M) until all the solid particles had dispersed; the EtOAc layer was separated, washed with water and dried (Na_2SO_4). The crude product left behind, after removal of solvent, was purified by column chromatography (Et_2O) to furnish sulfoxide ***R*)-91** (0.78g, 81%) as a white crystalline solid; $[\alpha]_D$ + 247 (*c* 0.1, acetone) (lit.[146] + 228, acetone).



(+)-(3*S*)-2-Methyl-1-(*R*-4-methylphenylsulfinyl)-1-phenylpropan-2-ol 166.

Bu^{t}Li solution in hexane (0.8 cm^3, 1.0mmol, 1.3M, hexane) was added to a solution of sulfoxide **(*R*)-91** (200mg, 0.87mmol) in anhydrous THF (20 cm^3) at – 70 ^oC. After leaving the reaction mixture stirred (0.5hr) at – 70 ^oC, dry acetone (1 cm^3) was added and the reaction mixture allowed to warm to room temperature; the reaction was quenched by the addition of a solution of NH_4Cl (25 cm^3, 10%). Most of the THF was distilled off under reduced pressure and the remaining liquid reaction mixture extracted with EtOAc (2 x 30 cm^3). The extract was dried (Na_2SO_4), solvent evaporated off and the crude product obtained was purified by PLC ($CHCl_3$) to yield sulfoxide **166** (165mg, 66%) as a colourless crystalline solid; mp 164 ^oC (from MeOH); R_f 0.30 (CHCl3); $[\alpha]_D$ +372 (*c* 0.4, $CHCl_3$) (Found: M^+-C_7H_7OS, 149.0965.

$C_{10}H_{13}O$ requires 149.0966); δ_H (500 MHz, $CDCl_3$) 1.29 (3 H, s, C*Me*$_A$Me$_B$), 1.77 (3 H, s, CMe$_A$*Me*$_B$), 2.28 (3 H, s, Ar*Me*), 3.32 (1 H, s, CH), 3.66 (1 H, s, OH), 6.96 (2 H, d, *J* 8.2, Ar), 7.02 (2 H, d, *J* 8.0, Ar), 7.11 (3 H, m, Ar), 7.19 (2 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 20.30, 28.29, 29.56, 73.28, 76.50, 123.17, 126.49, 126.87, 128.13, 129.69, 130.02, 136.86, 139.93; m/z (EI) 149 (M^+-C_7H_7OS, 97%), 139 (M^+-$C_{10}H_{13}O$, 44%), 91 (M^+-$C_{10}H_{13}O_2S$, 100%).



(+)-(2*S*)-2-(*R*-4-Methylphenylsulfinyl)-1,1,2-triphenylethan-1-ol 167.

Sulfoxide **(*R*)-91** (200mg, 0.87mmol), Bu^{t}Li solution (0.8 cm^3, 1.0mmol, 1.3M, hexane) and benzophenone **165** (230mg, 1.3mmol) were reacted following the procedure described for the synthesis of sulfoxide **166** to yield sulfoxide **167** (255mg, 71%) as colourless crystalline solid; mp 143 ^{o}C (from MeOH); R_f 0.45 (Et_2O); $[\alpha]_D$ +137 (*c* 0.7, $CHCl_3$); (Found: M^+, 412.1474. $C_{27}H_{24}O_2S$ requires 412.1497); δ_H (500 MHz, $CDCl_3$) 2.27 (3 H, s, Ar*Me*), 4.48 (1 H, s, CH), 5.86 (1 H, s, OH), 6.90 (2 H, d, *J* 8.2, Ar), 6.95-7.07 (10 H, m, Ar), 7.35 (3 H, m, Ar), 7.49 (2 H, m, Ar), 7.88 (2 H, d, *J* 7.4, Ar); δ_C (125 MHz, $CDCl_3$) 21.36, 74.79, 81.71, 124.33, 125.46, 126.17, 126.46, 127.30, 127.48, 127.65, 127.76, 128.70, 129.16, 130.41, 131.36, 137.10, 141.37, 144.97, 146.15; m/z (EI) 394 (M^+-H_2O, 0.25%), 287 (M^+-C_6H_6OS, 31%), 273 (M^+-C_7H_7OS, 22%), 256 (M^+-$C_7H_8O_2S$, 100%).

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